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Gene Transfer Studies in the Silkworm *Bombyx mori*

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ABSTRACT: The prebalstoderm stage silkworm eggs (3 h & 8 h ALL) were subjected to microinjection. The fate of the injected plasmid (pBSK+) DNA was monitored during the embryonic, larval/pupal, adult stages and subsequently in their progenies. Extrachromosomal persistence and transmission of the injected plasmid DNA was confirmed following slot blot, Southern bolt, plasmid rescue and PCR technique.
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KEYWORDS: *Bombyx mori*, microinjection, gene transfer, episomal persistence, transmission.

INTRODUCTION

Transgenesis is the modern technique adapted for the genetic improvement of livestock. It basically involves the transfer of foreign DNA into the fertilized eggs and integration of the transferred DNA into the genome of the host. Microinjection is an established technique for gene transfer in fish, birds, rodents, farm animals and insects (Maclean, 1994). In insects, *Drosophila* served as the model system for gene transfer studies. Various methods like microinjection (Tamura *et al.*, 1990; Nikolaev *et al.*, 1993), partical bombardment (Horard *et al.*, 1994), sperm mediation (Shamila and Mathavan, 1998a), electroporation (Shamila and Mathavan, 1998b) and pulse field electrophoresis (Zhengang *et al.*, 1997) have been adapted gene transfer studies in the silkworm *Bombyx mori*. Different types of vector DNA have been transferred into silkworm eggs to study their persistence/transmission.

In the present study the plasmid DNA pBSK+ was injected into the eggs of silkworm and the fate of the injected DNA during development is reported in this paper.

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FIGURE 1. Slot blot analysis of DNA extracted from embryonic stages of *B. mori* eggs, microinjected with pBSK+. Each slot was loaded with 2 μg of genomic DNA. Slot A1: pBSK+ (positive control: 1 μg/slot); Slot A2: DNA from uninjected eggs (negative control: 2 μg/slot); Slots A3–A11: Genomic DNA extracted from 1st–8th day developing eggs and freshly emerged larvae (3 h AEL); Slots B3–B10: DNA extracted from 1st–3rd & 5th–8th day developing eggs and freshly emerged larvae (8 h AEL).

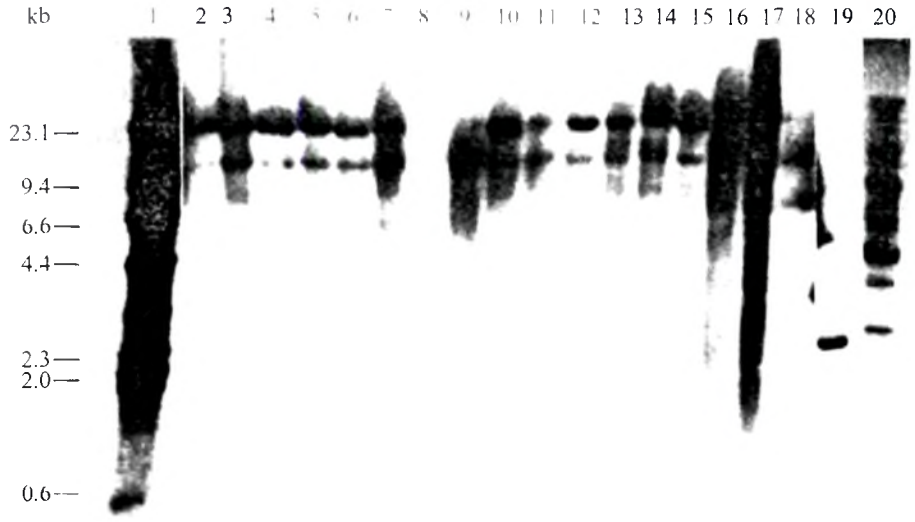

FIGURE 2. Southern blot analysis of the genomic DNA (undigested) extracted from eggs injected with pBSK+. Lane 1: Lambda/*Hind* III; Lanes 2–10: DNA from 1st–8th day developing eggs and freshly emerged larvae (3 h AEL); Lanes 11–18: DNA from 1st–5th & 7th–8th day eggs and freshly emerged larvae (8 h AEL); Lane 19: pBSK+/*Cla* I (positive control); Lane 20: pBSK+ undigested (positive control).

MATERIALS AND METHODS

The plasmid vector (pBSK+; 2958 bp) was used for microinjection. The vector DNA was amplified in *Escherichia coli* strain HB101, following alkaline lysis method and purified on CsCl/ethidium bromide gradient centrifugation (Sambrook *et al.*, 1989).

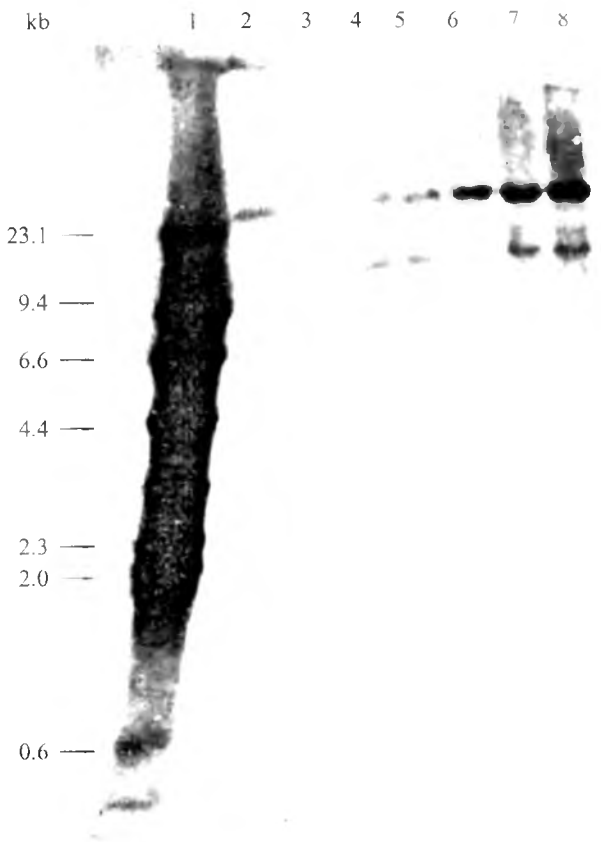

FIGURE 3. Southern blot analysis of the genomic DNA extracted from eggs injected with pBSK+. DNA was digested with *Bcl* I enzyme (*Bcl* I has no site in the plasmid vector). Randomly selected sample were subjected to Southern blot hybridization. Lane 1: Lambda/*Hind* III; Lanes 2–6: DNA from 1st, 3rd, 5–7th day eggs (3 h AEL); Lanes 7 and 8—DNA from 8th day and freshly emerged larvae (8 h AEL).

Silkworm eggs (Pure Mysore) collected 3 h and 8 h after egg laying (AEL) were microinjected with plasmid DNA (supercoiled; 500 μg/ml) dissolved in TE buffer (10 mM Tris/HCl pH 7.4; 1 mM EDTA). Simple manual method of injection as described by Shamila and Mathavan (1996) was followed for DNA transfer. Control eggs were injected with TE buffer alone. The microinjected eggs were kept at 10 °C for few minutes prior to incubation at 27 °C. The larvae emerged from the microinjected batches and their progenies were reared in the laboratory.

DNA was extracted from the microinjected eggs following the method of Jowett (1986). Genomic DNA was extracted every 24 hours from the microinjected eggs during the entire period of embryonic development and also from the freshly emerged larvae. DNA was extracted from somatic tissues as well as from reproductive tissues

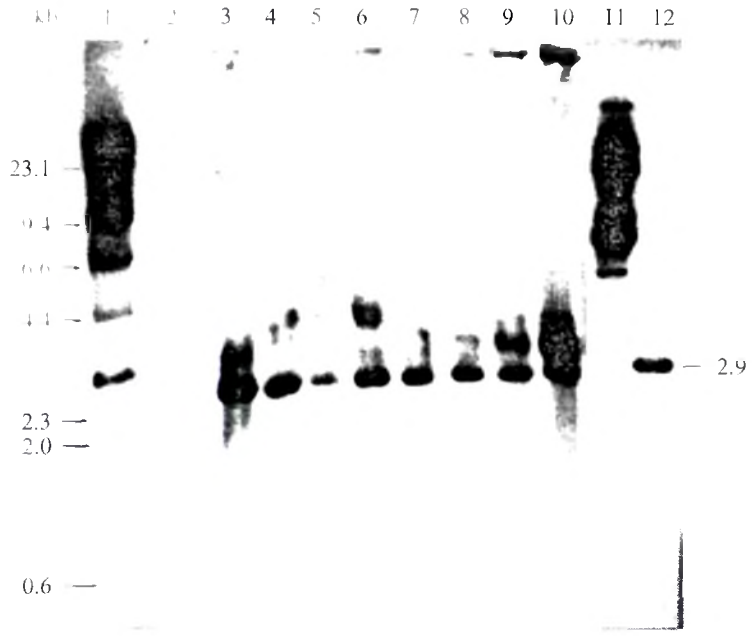

FIGURE 4. Southern blot analysis of DNA extracted from eggs injected with pBSK+. DNA was linearized with *Cla* I enzyme (*Cla* I has single site in the plasmid vector). Lane 1: λ/*Hind* III; Lane 2: DNA from uninjected eggs (negative control); Lanes 3–6: DNA from 2nd, 5th day developing eggs (3 h AEL); Lanes 7–10: DNA from 6th–8th day eggs and freshly emerged larvae (8 h AEL); Lane 11: pBSK+ undigested (positive control); Lane 12—pBSK+/*Cla* I (positive control).

of V instar larvae, pupae and moths of injected batch as well as from the control individuals.

For slot blot analysis, genomic DNA (2.0 μg) extracted from the experimental samples were transferred onto a nylon membrane using slot blot apparatus. This was then placed on whatman 1 filter paper pre-wetted with 0.4 M NaOH for 30 min to denature the DNA. For Southern blot analysis the DNA samples (digested or undigested) were electrophoresed on 0.9% agarose gel and transferred onto a nylon membrane by passive transfer method (Sambrook *et al.*, 1989). The DNA in the gels were depurinated (0.2 N HCl for 10 min), denatured (0.5 M NaOH, 1.5 M NaCl for 10 min, twice) and neutralized (0.5 M Tris HCl pH 8.0; 1.5 M NaCl for 15 min, twice) prior to transfer onto a nylon membrane. The DNA were fixed to the membranes by UV cross linking 1200 μj/cm for 90 sec.

Standard protocols were followed for prehybridization and hybridization of the membranes prepared following slot blot and Southern blots (Sambrook *et al.*, 1989). Probe DNA (pBSK+) was radiolabeled with α ^{32}P dCTP following random priming method as per manufacturer's instructions (Boehringer-Mannhem, Germany). After

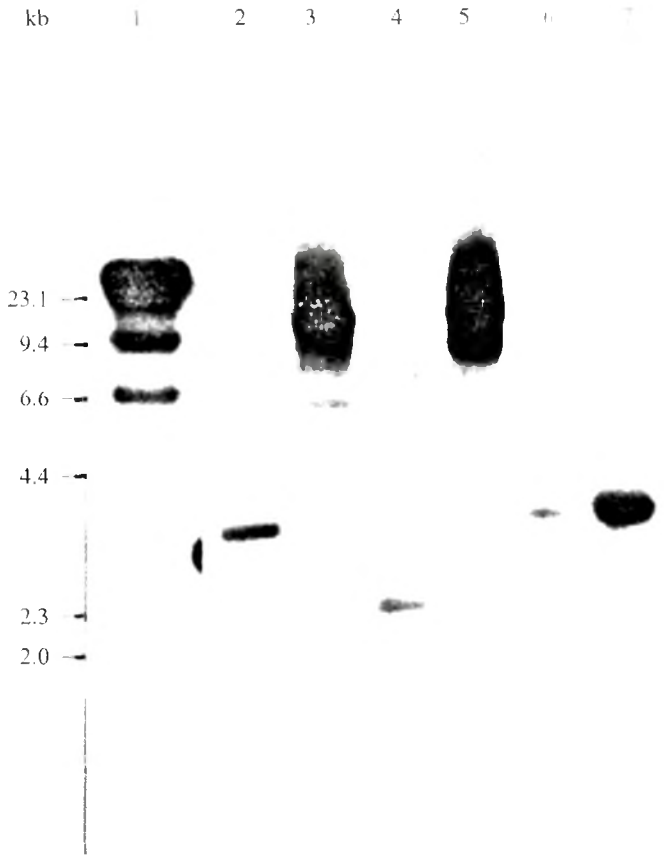

FIGURE 5. Southern hybridization of rescued plasmid from pBSK+ injected batch (3 h & 8 h AEL). Lane 1: Lambda/*Hind* III; Lane 2, 4, 6: rescued plasmids (from freshly emerged larvae) digested with *Hind* III; (Lane 4 appears to be partially digested); Lanes 3, 5: rescued plasmid undigested; Lane 7: pBSK+/*Hind* III (positive control).

hybridization the membranes were washed at high stringency (2X SSC and 0.1% SDS, at room temperature; 0.5X SSC and 0.1% SDS; 0.1X SSC and 0.1% SDS at 65 °C for 15 min each) and exposed to X ray film at −70 °C for desired periods to detect the hybridization signals.

For plasmid rescue experiment, an aliquot of the genomic DNA extracted from the larvae that emerged from the injected batch and also in their progeny were transformed in *E. coli* HB101. The plasmid DNA was reisolated from transformed colonies. The rescued plasmid DNA was characterized to confirm the persistence of the injected plasmid in the experimental individuals.

The Polymerase Chain Reaction (PCR) was also performed to check the persistence of injected sequences in the experimental samples. The following forward and reverse

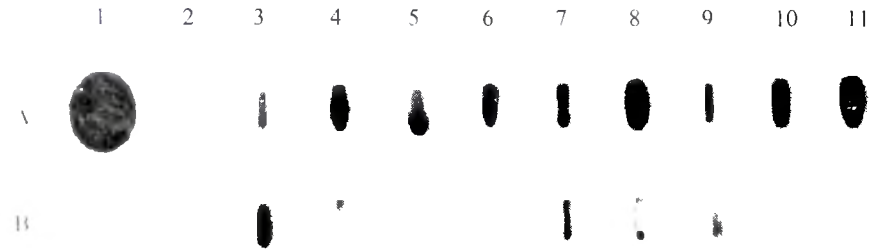

FIGURE 6. Slot blot analysis of DNA extracted from F_0 and F_1 test individuals. (enclosed from the eggs injected with pBSK+). Each slot was loaded with 15 μg of genomic DNA. Slot A1: pBSK+ (positive control; 2 μg); Slot A2: larvae enclosed from uninjected egg batch (negative control; 5 μg); Slot A3–A11: Different larval tissues from various instars; Slots B3–4: pupal tissues; Slot B5: ovary from pupae; Slot B6–B7: Reproductive tissues. Slot B8: eggs (F_1); Slot B9: freshly emerged larvae from eggs (F_1); Slot B10: III instar larval tissue (F_1).

primers were used. (1) forward primer M13: 5′GCCAGGGTTTTCCCAGTCACGA 3′; Reverse primer M13: 5′GAGCGGATAACAATTTCACACAGG 3′. PCR reaction was done in a final reaction volume of 50 μl; 0.1 μm forward and reverse primers; 1 X PCR buffer, (10 mM Tris –HCl (pH 9.0) 1.5 mM KCl; 2 mM $MgCl_2$; 0.1% Triton X-100); 300 ng genomic DNA as template; 0.05 mM dNTPs and 0.5 units Taq DNA polymerase. The reaction was for a minimum of 35 cycles consisting of 1 min at 95 °C and 1.5 min at 72 °C with final extension for 7 min at 72 °C. After the reaction, 10 μl of sample was mixed with bromophenol dye and analyzed in 2% agarose gel.

RESULTS

A total of 2453 eggs (3 h and 8 h AEL) were injected with the plasmid DNA (pBSK+). Genomic DNA was extracted from microinjected eggs (100 eggs/extraction) or freshly emerged larvae (25 larvae/extraction) and subjected to slot blot analysis (Fig. 1). Southern analysis of above test samples were presented in Fig. 2. The injected DNA persisted in different conformational forms. High molecular weight forms and covalently closed circular forms hybridized strongly with the probe where as relaxed and linearized forms hybridized weakly indicating the relative abundance of the forms (such as supercoiled, nicked, open dimers and multimers of high molecular weight forms). Genomic DNA samples were digested with *Bcl* I (*Bcl* I has no site in the injected plasmid DNA) and subjected to Southern analysis (Fig. 3). The pattern of hybridization was identical to that of hybridization reported in Fig. 2. Analysis of the hybridization data revealed the persistence of the injected DNA in the developing embryos and also in the freshly emerged larvae as unintegrated copy. DNA extracted from injected batch was digested with *Cla* I (*Cla* I has single site in the vector) and hybridized with pBSK+ probe; the hybridization signal at 2.9 Kbp indicate the

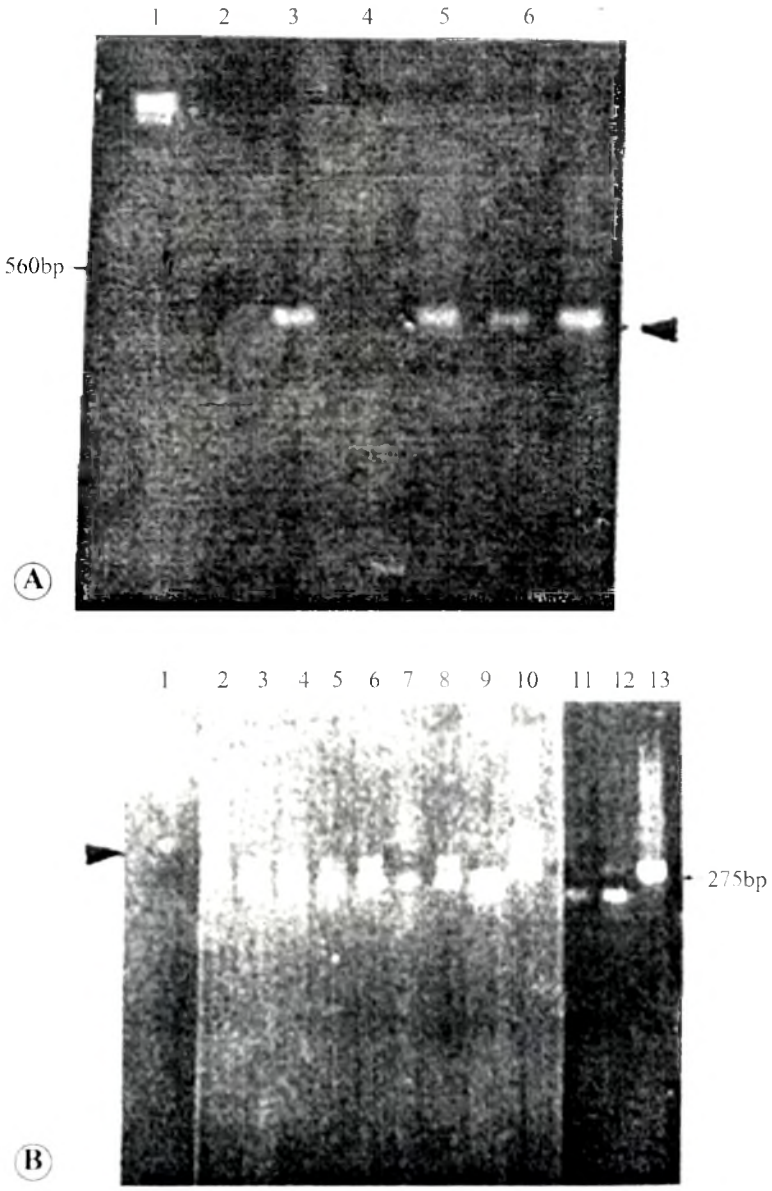

FIGURE 7. PCR amplification of internal sequences of pBSK+ persisting in the injected batches. (A) Lane 1: Lambda/*Hind* III; Lanes 2 & 4: DNA from uninjected eggs (negative control); Lane 3: DNA from 1st day eggs (3 h AEL). Lanes 5–7: DNA from 5th and 7th day developing eggs and also from freshly emerged larvae (8 h AEL). (B) Lane 1: Lambda/*Hind* III; Lane 2: III instar 3rd day larval tissues; Lane 3: IV instar 4th day tissues; Lane 4 V instar 6th day larval tissues; Lane 5: pupal ovary; Lane 6: female moth tissues; Lane 7: testis from moth; Lane 8: eggs (F_1); Lane 9: freshly emerged larvae (F_1); Lanes 10 & 13: pBSK+ (positive control); Lane 11: DNA from larvae that enclosed from uninjected batch (negative control); Lane 12: III instar larval tissue (F_1).

persistence of the injected plasmid without modifications (Fig. 4). However, the hybridization above the plasmid size suggest the partial digestion of the plasmid.

Episomal copies, of the plasmid DNA were rescued from the DNA extracted from freshly emerged larvae of the injected batch. Genomic DNA from the larvae was digested with *Hind* III enzyme, transformed in *E. coli* and screened for transformants. The plasmid DNA was extracted from transformed colonies showed identical restriction pattern as that of the injected plasmid (Fig. 5).

The *B. mori* larvae emerged from the microinjected eggs (random samples from 3 h, 8 h AEL injected batch) were reared in order to assess the persistence of the injected sequences in the larval stage. Moths emerged from the injected batch (F_0) were either self crossed or crossed with uninjected control and F_1 progenies were reared; DNA was extracted from the progenies and subjected to slot blot analysis to trace the persistence of the injected transgene (Fig. 6). From slot blot analysis it is evident that the injected DNA persisted in the progenies also. The above samples were subjected to Southern blot analysis; hybridization signal at 2.9 Kb corresponding to the plasmid (data not shown) confirmed that injected DNA persisted as unintegrated form. PCR was performed using the DNA template that was extracted from the microinjected eggs. If the plasmid DNA persisted as integrated copy the primers are expected to amplify a product about 275 bp (Fig. 7). Amplification of 275 bp PCR product again confirm the persistence of the injected DNA throughout the embryonic, larval development and progenies.

DISCUSSION

Microinjection of pBSK+ DNA into silkworm eggs (prebalstoderm stage) resulted in the persistence, extrachromosomal inheritance and transmission of the plasmid DNA to the next generation. However, the injected DNA failed to integrate with the host genome and it persisted as extrachromosomal copies without any modifications.

The persistence and transmission of high copy numbers of extrachromosomal persisting injected DNA (with or without replications) have been observed in other organisms, such as nematode—*Caenorhabditis elegans* (Stinchcomb *et al.*, 1985) mite—*Metaseiulus occidentalis* (Presnail and Hoy, 1992; Jeyaprakash *et al.*, 1998) insects—*Locust migratoria* (Mathi *et al.*, 1991); Drosophila (Martin *et al.*, 1986; Shen and Sofer, 1991); B. mori (Nikolaev *et al.*, 1993; Shamila and Mathavan, 1998a,b, 1999); Sea urchin (McMahon *et al.*, 1985); frog (Harland and Laskey, 1980); zebrafish (Stuart *et al.*, 1988).

The extrachromosomal persistence of the DNA in the silkworm may be due following reasons (i) unequal syncytial division of the embryo (Nagy *et al.*, 1994; Mathi *et al.*, 1991), (ii) formation of pseudonuclei (Steller and Pirrotta, 1985) and (iii) intranuclear localization (Etkin *et al.*, 1984).

Generally the genetic transformation of *Drosophila* was achieved through transposable element (*p*-element) based vectors (Rubin and Spradling, 1982). The transposable elements such as *mariner, Minos, Hermes, hobo, piggyBac*, have been identified and their use as transformation vectors have been reported (O'Brochta and Atkinson,

1996; Marshall, 1998). Though the present plasmid does not contain neither transposable elements nor repetitive sequence in the vector, the method demonstrate that the simple method used for introducing DNA through the hard chorion of the egg leads to stable transmission of foreign DNA into the germ cell lineage. Efforts are presently undertaken for the construction transformation vector based on transposable elements like *mariner, piggybac* for the stable integration of vector with *B. mori* genome for the production of transgenic silkworm.

ACKNOWLEDGEMENT

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REFERENCES

Etkin, L. D., Pearman, B., Roberts, M. and Bektesh, S. L. (1984) Replication integration and expression of exogenous DNA injected into fertilized eggs of *Xenopus laevis*, *Differentiation* **21**: 194–202.

Harland, R. M. and Laskey, R. A. (1980) Regulated replication of DNA microinjected into eggs of *Xenopus laevis*, *Cell* **21**: 761–771.

Horard, B., Mange, A., Pelissier, B. and Couble, P. (1994) *Bombyx* gene promoter analysis in transplanted silkgland transformed by partical delivery system, *Insect Mol. Biol.* **3**: 261–265.

Jowett, T. (1986) Preparation of nucleic acids, In: *Drosophila A Practical Approach*, Roberts, D. B.: (Editor). IRL Press Ltd: Oxford, 275–286.

Jeyaprakash, A., Lopez, G. and Hoy, M. A. (1998) Extrachromosomal plasmid DNA transmission and amplification in *Metaseiulus occidentalis* (Acari: Phytoseiidae) transformants generated by maternal microinjection, *Ann. Entnom. Soci. Amer* **91**: 730–736.

Maclean, N. (1994) *Animals with Novel Genes*, Cambridge University Press: New York.

Marshall, A. (1998) The insect are coming, *Nature Biotechnol.* **16**: 530–532.

Martin, P., Martin, A., Osmani, A. and Sofer, N. (1986) A transient expression assay for tissue specific gene expression of alcohol dehydrogenese in *Drosophila*, *Dev. Biol.* **117**: 574–580.

Mathi, S. K., Walker, V. K. and Wyatt, G. R. (1991) Expression from two *Drosophila* promoters in embryos of migratory Locust, *Insect Biochem.* **21**: 623–629.

McMahon, A. P., Flytzanis, C. N., Hough-Evans, B. R., Katula, K. S., Britten, R. J. and Davidson, E. H. (1985) Introduction of cloned DNA into sea urchin egg cytoplasm: Replication and persistence during embryogenesis, *Dev. Biol.* **108**: 420–430.

Nagy, L., Riddiford, L. and Kiguchi, K. (1994) Morphogenesis in the early embryo of the lepidopteran *Bombyx mori*, *Dev. Biol.* **165**: 137–151.

Nikolaev, A. I., Tchkonia, T. T., Kafiani-Eristavi, C. A. and Tarantul, V. Z. (1993) Preferential extrachromosomal localization of exogenous DNA in transgenic silkworm *Bombyx mori*, *L. Mol. Gen. Genet.* **236**: 326–330.

O'Brochta, D. A. and Atkinson, P. W. (1996) Transposable elements and gene transformation in Non-*Drosophilid* insects, *Insect Biochem. Molec. Biol.* **26**: 739–753.

Presnail, J. K. and Hoy, M. A. (1992) Stable genetic transformation of a beneficial arthropod, *Metaseiulus occidentalis (Acari: Phytoseiidae)* by a microinjection technique, *Proc. Natl. Acad. Sci. USA* **89**: 7732–7736.

Rubin, G. M. and Spradling, A. C. (1982) Genetic transformation of *Drosophila* with transposable element vectors, *Science* **218**: 348–353.

Sambrook, J., Fritsh, E. F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbour Laboratory Press: New York.

Shamila, Y. and Mathavan, S. (1996) A simple revised manual microinjection method of gene transfer into *Bombyx mori* eggs, *Indian. J. Seric.* **35**: 80–82.

Shamila, Y. and Mathavan, S. (1998a) Sperm mediated gene transfer in the silkworm, *Bombyx mori*, *Arch. Insect. Biochem. Physiol.* **38**: 168–177.

Shamila, Y. and Mathavan, S. (1998b) Gene transfer in silkworm, *Bombyx mori via* electroporation, *Biotechnol. Tech.* **12**: 507–510.

Shamila, Y. and Mathavan, S. (1999) Extrachromosomal transmission of microinjected DNA to the progeny of silkworm *Bombyx mori*, *Biotechnol. lett.* (press).

Shen, N. L. L. and Sofer, W. H. (1991) Introduction of single-stranded ADH genes into *Drosophila* results in tissue-specific expression, *Biochem. Biophys. Res. Com.* **174**: 1300–1305.

Steller, H. and Pirrotta, V. (1985) Fate of DNA injected into early *Drosophila* embryos, *Dev. Biol.* **109**: 54–62.

Stinchcomb, D. T., Shaw, J. E., Carr, S. H. and Hirsh, D. (1985) Extrachromosomal DNA transformation of *Caenorhabditis elegans*, *Mol. Cell Biol.* **5**: 3484–3496.

Stuart, G. W., McMurray, J. V. and Westerfied, M. (1988) Replication, integration and stable germ line transmission of foreign sequence injected into early zebrafish embryos, *Development* **103**: 403–412.

Tamura, T., Kanda, T., Takiya, S., Okano, K. and Maekawa, H. (1990) Transient expression of chimeric CAT genes injected into early embryo of the domesticated silkworm, *Bombyx mori*. *Jpn. J. Genet.* **65**: 401–410.

Zhengang, L., Rui, R. and Zhigang, Z. (1997) A new technique of transgenesis by PFGE, *Sericologia* **37**: 429–435.

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A Comparative Study on the Trehalose Level in Different Varieties of the Silkworm, *Bombyx mori*, During Fifth Instar Larval Development

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ABSTRACT. The level of Trehalose in the heamolymph of fifth instar larva of three different varieties of the silkworm *viz.*, Biovoltine (BV), Multivoltine (MV) and the cross bread (CB) was investigated. The trehalose level was very low on the first day in all the three varieties which increased with the development of fifth instar larva, attained the peak on the sixth day in case of BV and CB, on the seventh day in case of MV and decreased thereafter till spinning. The trehalose level was found to be maximum in BV followed by CB and MV. Interestingly the blood trehalose level was shown to be maintained at a more or less constant level during the active feeding period, i.e. from fourth day to sixth day, of the fifth instar development in all the three varieties. The results are discussed in relation to the food consumption and the regulation of trehalose level in the silkworm larva.
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KEYWORDS: *Bombyx mori*, bivoltine, multivoltine, crossbread, trehalose.

INTRODUCTION

Insects need carbohydrates as major fuel for their growth and development, being derived mostly from the diet. The silkworm, *Bombyx mori*, conserves sufficient quantity of energy reserve during larval stage to be utilized during pupal and adult stages. Trehalose is the major and metabolically active, nonreducing disaccharide in the insect blood (Wyatt and Kalf, 1956, 1957) which is synthesized in the fat body, (Candy and Kilby, 1959, 1961; Clegg and Evans, 1961) and utilized during spinning, flight and starvation of insects (Saito, 1960; Horie, 1961). It is well known that heamolymph, the only extracellular fluid in insect, is having diverse functions (Pawar and Ramakrishnan, 1977). It is the reservoir for most of the biochemicals that are required for nearly every physiological activity of the insect. Thus the change in the composition of heamolymph reflects the morphogenic and biochemical changes

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TABLE 1. Changes in the level of trehalose (blood sugar, mg/ml) in the haemolymph of V instar larva of the silkworm, *Bombyx mori* L.

Duration in days	Silkworm varieties		
	Bivoltine (NB_4D_2)	Multivoltine (PM)	Cross breed (CB)
1	3.788 ± 0.099	3.450 ± 0.000	3.602 ± 0.148
2	4.600 ± 0.000	3.680 ± 0.163	3.912 ± 0.130
3	7.314 ± 3.910	3.910 ± 0.000	5.104 ± 0.190
4	7.912 ± 0.126	4.830 ± 0.000	6.020 ± 0.247
5	8.280 ± 0.000	5.290 ± 0.163	8.082 ± 0.072
6	8.726 ± 0.139	5.566 ± 0.103	8.694 ± 0.192
7	8.280 ± 0.000	5.980 ± 0.000	7.222 ± 0.126
7	7.456 ± 0.148	5.340 ± 0.130	6.892 ± 0.102
CD-1 @ 5% = 0.8955			
@ 1% = 1.2555			
CD-2 @ 5% = 1.3678			
@ 1% = 1.9178			

CD-1: Comparison between silkworm varieties.
CD-2: Comparison between days.

taking place in insect tissues. The insect heamolymph performs several physiological functions such as immunity, transport, storage etc. (Mullins, 1985). In the present investigation, an attempt has been made to study the changes in trehalose level in the heamolymph during fifth instar development of different varieties of the silkworm, *Bombyx mori* L.

MATERIALS AND METHODS

Disease free layings of three different silkworm varieties *viz.*, Bivoltine (NB_4D_2), Multivoltine (Pure Mysore, PM) and the cross bred (PM × NB_4D_2) were obtained from NSSP Bangalore, and the eggs were incubated at a temperature of 25 °C and a relative humidity of 75%. After hatching, the larvae were reared under standard laboratory conditions on mulberry leaves (M5 variety). During young age rearing (up to III instar), the worms were maintained at a temperature of 26–28 °C and a relative humidity of 80–90%. However, during late age rearing (IV and V instar), the worms were maintained at a temperature of 24–26 °C and a relative humidity of 70–80% as recommended by Krishnaswamy *et al.* (1973). The fifth instar larvae were used for the experiment. The haemolymph was collected from the larvae in a clean prechilled test tube containing a few crystals of thiourea, by cutting the caudal horn (Kuwana, 1937). The heamolymph was centrifuged at 3000 rpm for 10 min. The supernatant was collected and used for the estimation of trahalose (blood sugar). Mentioned in the discussion by Phenol sulphuric acid method according to the procedure of Dubois

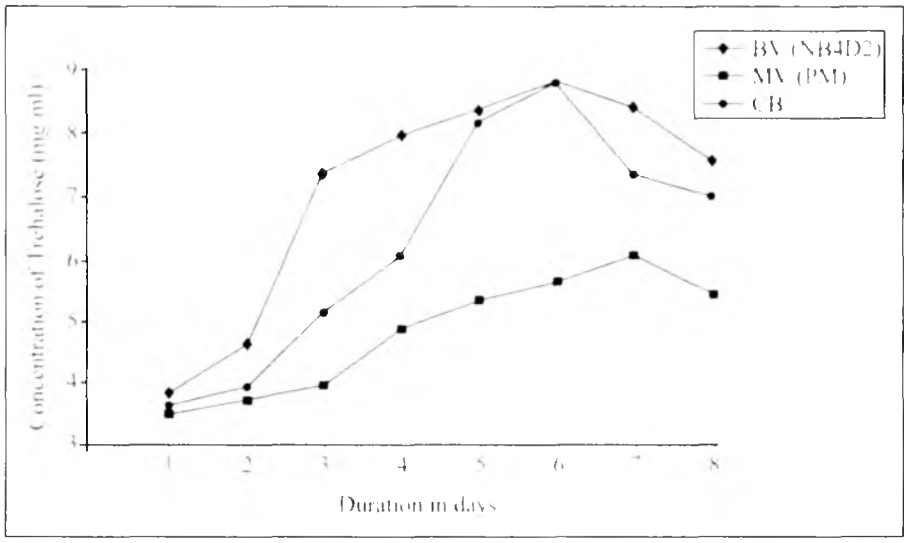

FIGURE 1. Changes in the level of Trehalose (Blood sugar) (mg/ml) in the haemolymph of the fifth instar larva of silkworm *Bombyx mori*.

et al. (1956) using glucose as standard. Four to Five worms were used for each data point. The mean value along with the standard deviation is presented in the results.

RESULTS

The heamolymph trehalose level was found to differ in all the three varieties during fifth instar larval development. It was found to be 3.60 mg/ml in BV, 3.60 mg/ml in CB and 3.45 mg/ml in MV on the first day. Thereafter a significant increase in the level was observed with advancement of age, to reach the maximum on the sixth day in BV (8.73 mg/ml) and CB (8.69 mg/ml), while on the seventh day in case of MV (5.98 mg/ml). The trehalose level decreased on the final day (spinning day) of fifth instar larval development. When compared between the varieties, the concentration of trehalose was maximum in BV (8.73 mg/ml) followed by CB (8.69 mg/ml) and MV (5.98 mg/ml) (Table 1, Fig. 1).

DISCUSSION

Trehalose is the major disaccharide in the insect heamolymph (Wyatt and Kalf, 1956, 1957). It's active biosynthesis in the fat body (Candy and Kilby, 1959, 1961; Clegg and Evans, 1961) and rapid utilization during flight and starvation of insects (Saito, 1960; Horie, 1961) has been reported clearly. The level of trehalose in the heamolymph reflects the level of carbohydrates in the body as well as the physiological status of the insect. The level of trehalose is found to be the lowest on the first day of fifth

instar in all the three varieties. This may be due to the fact that the tender mulberry leaves fed to the 'out-of-moult' worms contain more proteins and less sugars leading to the lowered consumption of carbohydrates (Krishnaswamy *et al.*, 1973; Benchamin and Nagaraj, 1987). The increase in the trehalose level with the development of fifth instar larva is perhaps due to the feeding of coarse leaves, which are rich in carbohydrates and as well as the higher rate of food consumption by the larva resulting in increased absorption of glucose through the digestive tract (Horie, 1959) to cope with the increased physiological activities. It has been reported that the heamolymph trehalose level in insects is maintained by the absorption of digested sugars through the gut (Horie, 1959) and/or by the break down of fat body glycogen to glucose, which serve as precursors for the synthesis of trehalose (Candy and Kilby, 1959, 1961; James *et al.*, 1961). Clegg and Evans (1961) reported the active biosynthesis of trehalose in the fat body which are released in to the heamolymph. Thus the peak level of trehalose observed on the sixth day in BV and CB and on the seventh day in MV may be associated with active depletion of fat body glycogen along with the increased absorption of sugars through the gut.

The decrease in the level of trehalose on the final day of fifth instar is the result of its greater utilization to furnish fuel for active synthesis of silk and other physiological processes like spinning (Evans and Dethier, 1957; Clegg and Evans, 1961). Further it may also be associated with the decreased food intake during the last part of the fifth instar.

Among the varieties studied, the trehalose level is found to be high in BV followed by CB and MV. This may perhaps be due to higher physiological activities such as food intake, digestion, assimilation and conversion in BV compared to CB and MV (Gururaj, 1995). Further the level of trehalose is found to be maintained at, more or less, a constant level between fourth and sixth day of fifth instar development in BV and MV, where as in case of the CB the level was maintained between fifth and sixth day, thereby maintaining the homeostasis (Saito, 1960, 1963) probably at the expense of fat body glycogen. Hirano and Yamashita (1980) have shown a correlation between the utilization rate and turnover rate of haemolymph trehalose during metamorphosis in *B. mori*. Thus the present results are in accordance with the earlier findings of Saito (1963) suggesting the existence of a possible homeostasis mechanism in the silkworm to regulate the trehalose level in the body.

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REFERENCES

Benchamin, K. V. and Nagaraj, C. S. (1987) Appropriate sericulture techniques, *Silkworm rearing techniques* 63–106.

Candy, D. J. and Kilby, B. A. (1959) Site and mode of trehalose biosynthesis in locust, *Nature London* **183**: 1594–1595.

Candy, D. J. and Kilby, B. A. (1961) The biosynthesis of trehalose in the locust fat body, *J. Biochem.* **78**: 531–536.

Clegg, J. S. and Evans, D. R. (1961) Blood trehalose and flight metabolism in the blowfly, *Science* **134**: 54–55.

Dubois, M., Gilles, K. A., Hamilton, J. H., Rebers, P. A. and Smith, F. (1956) Colorimetric micro method for determination of sugars and related substances, *Anal. Chem.* **28**: 350–356.

Evans, D. R. and Dethier, V. G. (1957) The regulation of taste thresholds for sugars in the blowfly, *J. Insec. Physiol.* **1**: 3–17.

Gururaj, C. S. (1995) Studies on the physiology and biochemistry of NPV infested silkworm *Bombyx mori* L. *Ph. D thesis*, Bangalore University.

Hirano, M. and Yamashita, O. (1980) Change in utilization rate and turnover rate of haemolymph trehalose during metamorphosis of *Bombyx mori*, *J. Seric. Sci. Japan* **49**: 506–511.

Horie, Y. (1959) Blood trehalose and fat body glycogen in the silkworm *Bombyx mori*, *Nature* **188**: 583–586.

Horie, Y. (1961) Physiological studies on the alimentary canal of the silkworm, *Bombyx mori*. III. Absorption and utilization of carbohydrates, *Bull. Seric. Exp. Jpn.* **16**: 287–309.

James, S., Clegg, R. and Evans, David (1961) Blood trehalose and flight metabolism in the blowfly *Phormia regina*, *Science* **134**: 54–55.

Krishnaswamy, S., Narasimhanna, M. N., Suryanarayana, S. K. and Kumararaj, S. (1973) Sericulture manual II—Silkworm rearing. Food and Agriculture Organisation of the United Nations. Rome. 131.

Kuwana, Z. (1937) Reducing power of the body fluid of the silkworm, *Jap. J. Zool.* **7**: 265–275.

Mullins, D. E. (1985) Chemistry and physiology of the haemolymph. In: *Comprehensive Insect Physiology and Pharmacology*, Vol. 3. Kerkut, G. A. and Gilbert, L. F.: (Editors). Pergamon press: 355–400.

Pawar, V. M. and Ramakrishnan, N. (1977) Biochemical changes in Larval haemolymph of *Spodoptera litura* fibricius due to nuclear polyhydrosis virus infection, *Indian J. Expt. Biol.* **15**: 755–758.

Saito, S. (1960) Trehalase of the silkworm *Bombyx mori*, purification and properties of the enzyme, *J. Biochem, Tokyo* **48**: 101–109.

Saito, S. (1963) Trehalase in the body fluid of the silkworm, *Bombyx mori*, *J. Insect Physiol.* **9**: 509–519.

Wyatt, G. R. and Kalf, G. F. (1956) Trehalase in insects, *Fed. Proc.* **15**: 188.

Wyatt, G. R. and Kalf, G. F. (1957) The Chemistry of insect haemolymph. I. Trehalose and other carbohydrates, *J. Gen. Physiol.* **40**: 833–847.

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Observations on Laboratory Mass Multiplication of Braconid Endoparasitoid *Apanteles taragamae* Wilk. On Early Instar Caterpillars of *Opisina arenosella* Walker on Coconut

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ABSTRACT: The procedure for mass multiplication of *Apanteles taragamae* on early instar caterpillars of *Opisina arenosella* is described. Egg to adult period of *A. taragamae* is completed in 17.92±3.41 (female) and 19.80±2.93 (male) days. Longevity of adults were 10.73±4.92 and 11.20±5.04 for male and female respectively. Maximum percentage parasitism occurs on the first 2 days of exposure of host larva although egg laying continued up to 15 days. Average percentage parasitism was 64.7 and 61.8% respectively under lab. and field conditions. 2000 Association for Advancement of Entomology

KEYWORDS: *Opisina arenosella*, *Apanteles taragamae*, coconut, endoparasitoid, lab mass multiplication.

INTRODUCTION

The black headed caterpillar pest of coconut palm *Opisina arenosella* Wlk. is attacked by 40 parasitoids and 20 predators during its different stages of growth (Pillai and Nair, 1993). Among these the indigenous parasitoids particularly the late larval parasitoid *Goniozus nephantidis* Mues. (Bethylidae), pre-pupal parasitoid *Elasmus nephantidis* Roh. (Elasmidae) and pupal parasitoid *Brachymeria nosatoi* Habu (Chalcididae) play a significant role in suppressing 94% population of *Opisina* on the coastal and backwater tracts of Kerala (Sathiamma *et al.*, 1987, 1996). These parasitoids are effective towards the advanced larval to pupal stages when the pest would have caused enough depredations to the coconut foliage. *Apanteles taragamae* Wilk. is a solitary endoparasitoid parasitising the early instar caterpillars of *O. arenosella*. Release of early larval or egg parasitoids would be more advantageous in checking the pest population at the initial stages of pest build up and before causing injury to the leaves.

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With this in view trials were initiated to mass culture *A. taragamae* in the laboratory so that a continuous supply of the parasitoid is ensured before commencing the field release and evaluation.

Of the genus *Apanteles*, species *taragamae* is recorded from *O. arenosella*. This parasite occurs in abundance in many coconut growing tracts of Kerala and its biology, nature of parasitism and hyperparasitism were recorded (Ghosh and Abdurahiman, 1985a, 1988, 1993). Very little attempts have been made for the mass rearing of this parasitoid and its successful utilization as a potential biocontrol agent. The present paper summerises the result of observations on the mass multiplication of this parasitoid in the laboratory.

MATERIALS AND METHODS

Observations were made in the laboratory at a temperature range of 26–33 °C and relative humidity of 77–93%.

Maintenance of host culture

Opisina host culture was maintained from moths obtained from field collected late larva/pupa. The moths were released on leaves of potted west coast tall coconut seedlings reared under insect proof field cages. Before releasing the months, bits of larval gallery or infested leaf bit with gallery were glued to the leaf of the seedling. The frass materials act as a strong attractant for the egg laying moths. The leaves are then individually caged in cylindrical insect proof nylon net cages (90 cm long and 40 cm wide) with a 10 cm long opening fitted with a zib on one side and a mouth (Fig. 1). The mouth of the cage is safely closed by tieing it to the petiole with a thread. Ten to fifteen newly emerged male and female moths (1 : 5) are released into each cage through the zib opening. After 18–21 days of moth release; suitable larvae can be collected from the cage for maintenance/multiplication of *A. taragamae*. Several such units are maintained for ensuring an adequate and un-interrupted supply of second instar host larvae.

Parasite culture

The stock culture of *A. taragamae* was obtained from field collected parasitoid cocoons and parasitised host larvae. Newly emerged adults were transferred to 250 ml conical flasks which are the egg laying units or cages. Adult parasitoids are fed on honey provided as tiny droplets on wax coated paper bits.

The larval galleries are gently tapped with a fine feather or a fine camel hair brush to excite and move them out of their galleries. Five to seven numbers of these larvae are then transferred to 4–5 cm long fresh green coconut leaf bits or on partially infested leaf bit with gallery and each bit were kept in the egg laying units (250 ml conical flask). The mouth of the flasks are then closed with muslin cloth or cotton plug.

The inoculated flasks are kept horizontally and undisturbed for 12 hours to allow the host larvae to settle down in the gallery in the leaf bit and start feeding on the leaf.

FIGURE 1. *Opisina* culture maintained on potted coconut seedling under caged condition.

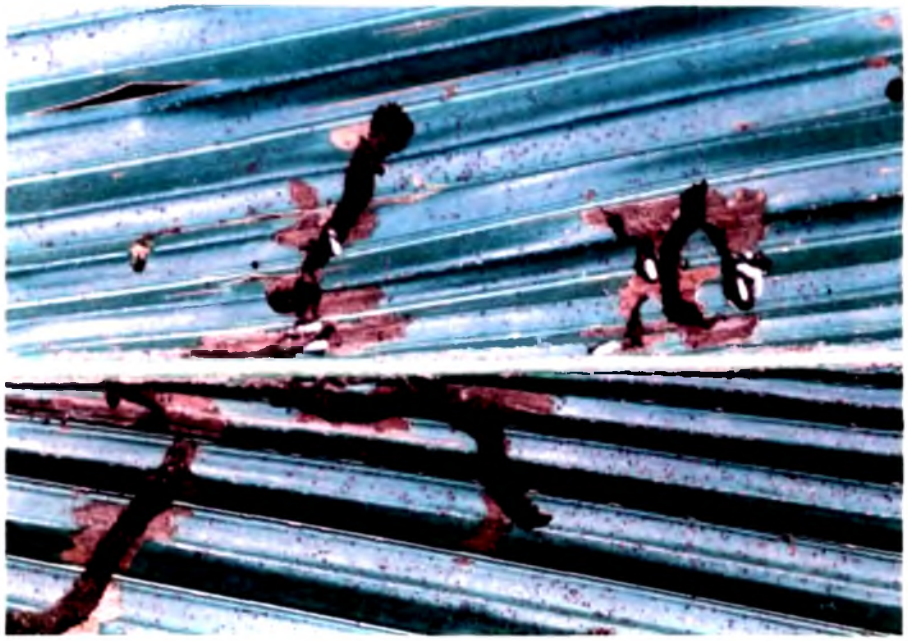

FIGURE 2. *Apanteles taragamae* cocoons on *Opisina* early instar larval gallery.

TABLE 1. Development particulars of *A. taragamae* in the laboratory

Particulars		No. of replications	Mean±SD	Range
Egg to adult	Male	50	19.80 ± 2.93	10–25
Period (days)	Female	50	17.92 ± 3.41	11–23
Larval period (days) (oviposition to final instar larvae)		20	9.80 ± 2.04	6–13
Longevity	Male	30	10.73 ± 4.92	3–21
	Female	30	11.20 ± 5.04	3–22
Oviposition period (days)		10	11.00 ± 4.06	4–15
Number of progeny	Total	10	16.20 ± 9.48	6–30
produced	Male	10	9.10 ± 4.65	2–19
	Female	10	8.90 ± 5.36	1–17

Laboratory scale mass multiplication

To evaluate the performance of *A. taragamae* under field conditions, mass multiplication of the parasitoid in the laboratory is a pre-requisite. For this purpose basic data on the duration of egg to adult stage, number and sex of progeny produced, oviposition period, percentage parasitism under laboratory were collected. The parasitoids were evaluated under field cage conditions.

Daily parasitism by *A. taragamae* was studied by exposing 20 *Opisina* larvae daily per female parasitoid. The exposed larvae were removed after 24 hr and were maintained separately. For this purpose they were transferred to fresh green leaf bits kept in the glass tubes 25 × 100 mm. The parasitised larvae feed on the leaf bit. Depending on the feeding activity of the larvae, the leaf bit is changed every alternate day till the parasitoid pupates. Cocoons were collected for further observations. 20 replications, each consists of a pair of freshly emerged male and female parasitoid, were maintained for recording this data.

To study the percentage parasitism under laboratory condition early larvae of *Opisina* with gallery were exposed into the egg laying chamber (250 ml conical flask) for parasitism and were removed after 24 hr of exposure. The parasitised larvae were maintained on fresh coconut leaf bits in test tubes changing the food daily or on caged coconut seedlings and cocoons were collected to record percentage parasitism. 30 replicates were maintained to record observations.

To evaluate parasites under field cage conditions *Opisina* moths were released in potted coconut seedlings. Number of early larvae were counted on 17–20th day of moth release and *Apanteles* were released in side the cage. Cocoons were collected and percentage parasitism recorded. 20 replications were maintained to record the data. To record natural parasitism and hyperparasitism monthly samples were collected (50 coconut leaflets collected at random) from a *Opisina* infested field of 0.5 ha at Thodiyoor, Kollam Dist. S. Kerala.

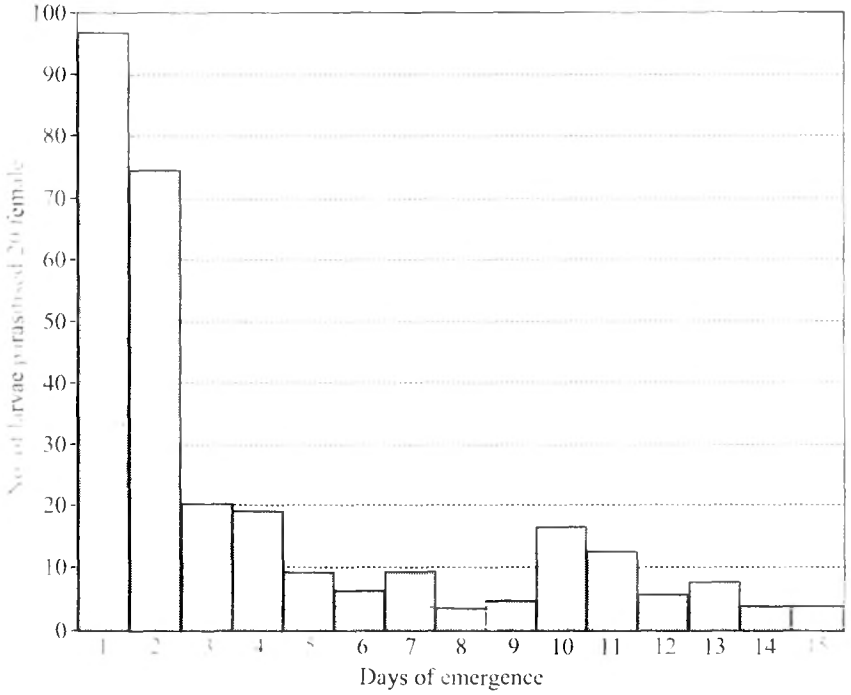

FIGURE 3. Daily parasitism by *A. taragamae* on *Opisina*.

TABLE 2. Percentage parasitism by *A. taravamae* under laboratory conditions

Total number of replications	30
Total number of larvae exposed	1271
Total number of larvae parasitised	822
Average percentage parasitism	64.7 (24.0–100.0)
Total number of adult parasitoids emerged	524
Average percentage of adults emerged	63.7 (33.3–92.3)

Figures given in the parenthesis represent the range for the different samples.

RESULTS AND DISCUSSION

The parasitoids mate soon after emergence and female is receptive throughout its life. Ovipositional activity is very high during the early hours of the day. Soon after introduction of the leaf bit containing the second instar host larvae, the parasitoid start searching for the host and when it comes in contact with the host, oviposition takes place. Due to occassional poking with the ovipositor, the larvae comes out of its gallery and roams inside the rearing chamber and the parasitoid chases and host and oviposites in it.

TABLE 3. Percentage parasitism by *A. taragamae* on *Opisina* under caged coconut seedlings

Total number of replications	20
Total number of larvae exposed	923
Total number of larvae parasitised	329
Average percentage parasitism	35.6 (25.0–44.2)

Figures in paranthesis represent the range value.

The parasitised host larvae is distinguished by its creamy/light yellow colour and a glossy external appearance. As it grows feeding is considerably reduced but it never enters the next larval stage. The larval parasitoid completes its development inside the host larva and when growth is completed it comes out of the host's body in about 10 days of oviposition. This parasite larva starts spinning cocoon and pupates in about 12 hr. Cocoons are white, elongated and cylindrical in shape (Fig. 2). Egg to adult period is completed In 17.92 ± 3.41 (range and 11–23) and 19.80 ± 2.93 (range 10–25) for females and males respectively. The shortest period observed is 10 days and longest 25 days (Table 1) Maximum number of adults emerges on the 20th day of oviposition. Adult parasites are fed immediately after emergence, on honey doplets placed on wax coated paper stripes. If food is not given immediately they die in 8–12 hours. Feeding lasts for 45–70 seconds. Ovipositing females feeds every now and then. Males live for 10.73 ± 4.92 (range 3–21) and females 11.20 ± 5.04 (range 3–22) days. Oviposition period of the female is 11.00 ± 4.06 (range 4–15) days. The total number of progeny produced per female is 16.20 ± 9.48 (range 6–30) comprising male progeny of 9.10 ± 4.65 (range 3–19) and female progeny of 8.90 ± 5.36 (range 1–17) (Table 1).

Observations of the number of the larvae parasitised by female at 24 hour interval is given in (Fig. 3). Data revealed that maximum parasitism of 24.25% (97/400 larva tested) occurred on the first day of exposure followed by 18.5% on 2nd day (74/400 larvae). Parasitism was only 5% or even less from the third day onwards and the oviposition period continued upto 15 days of exposure. No parasitism was observed beyond 15 days.

Studies on percentage parasitism by *A. taragamae* in the laboratory revealed that the parasitism effected is 64.7% (822/1271 *Opisina larvae* exposed) and it varied from 24.0 to 100%. 12.4% of the larvae escaped stinging/egg laying by the parasite and they developed as normal larvae; whereas, 23% of the larvae died either by stinging by the parasite or by injury while handling or by laying eggs and the parasite failed to develop. Percentage of adult parasites emerged from the parasitised larvae is 63.7% (524/822 parasitised larvae (Table 2)).

Parasitism was also studied on caged seedlings maintained under field cage. Parasitism observed was 35.6% (329/923 *Opisina* larvae tested) and it varied from 25.0 to 44.2% (Table 3).

Evaluation of natural parasitism in the field revealed that mean percentage parasitism was 61.8 (320 cocoons/518 early larvae collected) Parasistism observed is quite

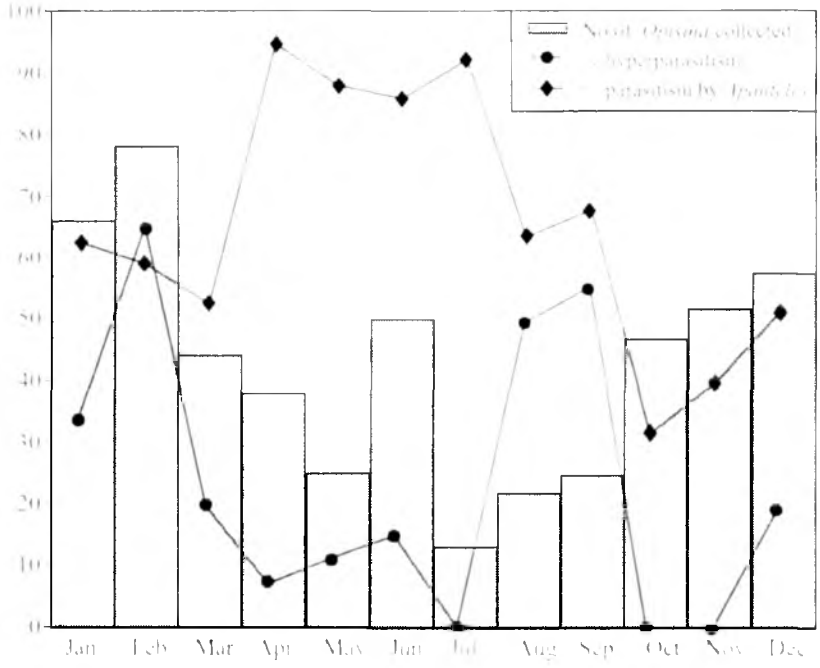

FIGURE 4. Field parasitism by *A. taragamae* on *Opisina* and hyper parasitism on cocoons of *A. taragamae*.

high with peak occurrence in April (94.7%) and October recorded a low percentage (31.9%). an important observation made from field collection of *A. taragamae* cocoons is that of a high percentage of hyperparasitism and it varied from 0 to 64.7% during different months (Fig. 4). The important hyperparasites recorded are species of *Pediobius*, *Eurytoma albotibialis* and *Brachymeria nephantidis*. Hyperparasitism occurring in nature limits the multiplication of *A. taragamae* and control of early larvae of *Opisina* in the field.

Ghosh and Abdurahiman (1993) reported that factors such as atmospheric temperature, host nurishment and host larval age effects the developmental period of *A. taragamae*. The shortest developmental period reported by Ghosh and Abdurahiman (1988) is 15 days in hot months ad 24 days in cold weather. The present study revealed 17 to 19 days as egg to adult period under 26–33 °C and 77–95% RH. Hyper parasites of *A. taragamae* reported by Ghosh and Abdurahiman (1985a,b) from various parts of north Kerala from random sampling were *Aphanogmus manilae* Ashmead accounting for 27.14% followed by *Meteroidae hutsoni* Nixon 17.14%, *Brachymeria nephantidis* Gahan and *Pediobius imbreus* Walker 2.86% each and *Eurytoma albotibalis* Ashmead 1.42%. Average percentage parasitism observed in the present study by monthly sampling from Thodiyur, Kollam Dist. South Kerala is 6.43% (296 parasitoids in 4606 host

larvae collected) which is very low when compared to the North Kerala conditions of 61.8%.

The present studies indicated that although oviposition period is upto 15 days maximum number of eggs were laid within the first four days of exposure of the host larvae.

Laboratory scale mass multiplication of *Apanteles* was attempted in the present paper and 64.7% parasitism was effected. This shows that the parasite is amenable for lab. rearing. The comparatively lower percentage of parasitism of 35.6 under field cage may be due to unavailability of suitable stage of the *Opisina* larvae for the parasitoid throughout its life in the caged condition. The nature of parasitism by *A. taragamae* is peculiar, in which the host larvae remain alive and feed even after parasitism. Daily supply of fresh food to the host larvae is difficult. So this phenomenon can be considered by relasing the parasitised host larvae to caged potted seedlings and subsequently collecting cocoons for field release. Evaluation of parasitism was done under field cages where as the earlier workers reported only average field parasitism from random samples collected from infested field. The present study also indicates the monthly fluctuations in the field population of *Apanteles taragamae* and hyperparasites in relation to the early instar of *Opisina* larvae.

REFERENCES

Ghosh, S. M. and Abdurahiman, U. C. (1985a) Bioethology and evaluation of comparative efficiency of *Apanteles taragame* a larval parasitoid of the coconut caterpillar *Opisina arenosella*, *Philippines J. Cocon. Stud.* **10**: 4–8.

Ghosh, S. M. and Abdurahiman, U. C. (1985b) On some hyperparasitoids of *Opisina arenosella*, the black headed caterpillar pest of coconut in Malabar, India, *Cocos* **3**: 29–31.

Ghosh, S. M. and Abdurahiman, U. C. (1988) Biology of reproduction of *Apanteles taragamae* Wilk. (Hym: Braconidae) a larval parasitoid of *Opisina arenosella* walker, the caterpillar pest of coconut, *Entomon* **13(2)**: 147–155.

Ghosh, S. M. and Abdurahiman, U. C. (1993) Variations in the developmental period during adverse conditions in *Apanteles taragamae* Wilkinson (Braconidae: Hymenoptera), *Ann. Entomol* **11(2)**: 27–31.

Pillai, G. B. and Nair, K. R. (1993) A checklist of parasitoids and predators of *Opisina arenosella* wlk on coconut, *Indian Cocon. J.* **23(9)**: 2–9.

Sathiamma, B., Pillai, G. B., Abraham, J., Bhat, K. S., Jayapal, S. P. and Nair, K. R. (1987) Norms for release of larval, pre-pupal and pupal parasitoids of *Opisina arenosella* wlk, the leaf eating caterpillar of coconut palm, *J. Plantn Crops.* **15**: 118–122.

Sathiamma, B., Sabu, A. S. and Pillai, G. B. (1996) Field evaluation of the promising species of indigenous parasitoids in the biological suppression of *Opisina arenosella* walker, coconut leaf eating caterpillar, *J. Plantn Crops* **24(1)**: 9–15.

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A Potent Kairomone for the Management of Sweet Potato Weevil *Cylas formicarius* F.

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ABSTRACT: Laboratory and field experiments were conducted to study the efficacy of dichloromethane extract of sweet potato periderm in attracting sweet potato weevils. Weevil attraction increased with concentration of crude extract. Laboratory studies showed that with 100 mg of pure compound, the attraction was 60.88%. Field studies conducted with crude extracts from different varieties revealed that the number of weevils attracted varied with varieties indicating the concentration of compound varied in different varieties. The attraction was maximum in variety RS-III-2. The pure compound attracting both male and female weevils was isolated from periderm and the IR spectral data and GC profile of the compound established the compound as boehmeryl acetate, a pentacyclic triterpenoid. The ratio of male female weevils attracted ranged from 2 to 7 : 1. Duration of biological activity of the crude extract and pure compound was in the range of 18–22 days.
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KEYWORDS: Sweet potato weevil, Kairomone, *Cylas formicarius*, Boehmeryl acetate, Management.

INTRODUCTION

Sweet potato, *Ipomoea batatas* (L.) is a major tuber crop grown extensively throughout the tropical and subtropical zones. *Cylas formicarius* F. is one of the formidable and destructive pests of sweet potato, world wide, both in field and in storage (Palaniswami, 1994). In Southern USA the loss attributable due to weevil exceeded US $7 million per year (Sorenson, 1984). On-farm survey in Kerala State of India revealed an annual yield loss to a tune of Rs. 9.6 million due to weevil (Palaniswami *et al.*, 1991). The prohibitive cost of insecticides and high application cost of the recommended crop protection measures deterred its wide adoption among sweet potato farmers. Use of synthetic sex pheromone trap has become increasingly more popular in IPM of sweet potato weevil. Though sex pheromone is a successful tool against *C. formicarius*, it attracts only male weevils (Wilson *et al.*, 1991). Sweet

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potato tubers as a source of attractant to adult *C. formicarius* was indicated earlier (Palaniswami and Mohandas, 1994). Agelopoulos *et al.* (1999) and Hall (1998) have highlighted use of semiochemicals for monitoring as well as for control of insect pests. Attraction of the weevil may be due to certain chemicals. An attempt was made to study the compound present in the periderm of sweet potato and its efficacy in attracting male and female weevils.

MATERIALS AND METHODS

Weevils were reared on tubers kept in glass jars at ambient temperature (28 to 32 °C) and RH (67 to 72 %). Freshly emerged adult weevils were transferred to fresh sweet potato tubers for egg laying and then the weevils were removed and tubers were kept for four weeks. The freshly emerging weevils after four weeks were used for all laboratory studies in order to make sure that same population was used in all experiments. Sweet potato varieties Kanhangad, Sree Bhadra, RS III-2 and 56-2, used in the studies, were raised in field following the package of practices and harvested after 105 days.

Extraction of sweet potato periderm

Extraction of sweet potato periderm was carried out following the procedure of Son *et al.* (1990). Sweet potato periderm (1–2 mm thickness) was peeled from the tubers and cut into small pieces and extracted with dichloromethane (AR) in soxhlet apparatus for 6–8 hours till an oily layer separated out. The two layers were separated using separating funnel. The dichloromethane soluble layer was filtered through anhydrous sodium sulphate and the solvent was removed by distillation. The crude extract was weighed and redissolved in dichloromethane for further studies.

For purification of crude extract one gram of crude extract was subjected to column chromatography over 40 g silica gel (60–120 mesh). Column was eluted with hexane (100%) and mixtures of hexane and ethyl acetate of increasing polarity. The different solvent mixtures of hexane:ethyl acetate 98 : 2, 96 : 4, 94 : 6, 92 : 8, 90 : 10 and 80 : 20 were used. Total volume of each solvent mixture was 200 ml. From each solvent mixture 10 ml fractions was collected. The various 10 ml fractions obtained were combined on the basis of TLC. These fractions were tested for attraction of weevil in the laboratory. The compound collected in the 90 : 10 fraction was further purified by recrystallisation.

Crude dichloromethane extract and the pure compound obtained after recrystallisation were analysed using Perkin-Elmer Gas Chromatograph, with 0.25 mm id × 30 m PE-5 silica column, with the temperature programme 150–300 °C at 5 °C/min, 30 min hold at 300 °C; flow rate 54 cm sec^{-1}, split flow rate 46 ml/min., H_2 injection port temperature 330 °C and FID temperature 400 °C.

Laboratory evaluation of crude extract

Efficacy of crude extract of Kanhangad variety was tested for its ovipositional stimulus and its attraction to sweet potato weevil. Different concentrations ranging from (2

to 100 mg) of the crude extract of Kanhangad variety were prepared and absorbent cotton was dipped in the extract, kept for vapourization of solvent. This cotton was covered with black cloth and kept in a glass chamber. Twenty five adult female weevils of the same age group (1 week old) were introduced into this chamber. The chamber as a whole was then covered with black cloth. Number of eggs laid by female weevils was counted after 48 hours. The entire experiment was repeated using different concentrations of the crude extract.

Crude extract from Kanhangad variety was tested for the attraction of weevils in the laboratory. 200 adult weevils of uniform age with 1 : 1 sex ratio were released in the laboratory (20 m × 20 m). A tin trap designed for pheromone studies in the Institute was used in the study. The cotton soaked in the crude extract was kept for 10 minutes to vapourise the solvent and then covered with black cloth for using in the trap. The trap was kept at the centre of the laboratory. After 24 hrs the number of weevils trapped was recorded. The entire experiment was repeated with different quantity of the crude extract (25, 50 and 100 mg).

Field evaluation of the crude extract

Crude extracts of four varieties Kanhangad, RS-III-2, Sree Bhadra and 56-2 were tested for attraction of sweet potato weevil in field. Four traps containing the crude extract (25 mg) of Kanhangad variety were kept 3 m apart in weevil infested sweet potato field. Number of weevils trapped was recorded after two days. The entire experiment was repeated with 50 and 100 mg of the extract and also using crude extracts of other three varieties, each at 25, 50 and 100 mg doses.

Efficacy of pure compound on attraction of weevil

Laboratory assay was conducted using pure compound for its attraction as described under laboratory evaluation of crude extract at three doses (25, 50, 100 mg).

Field evaluation of the pure compound was carried out as described under field evaluation of crude extract at different doses (25, 50, 100 mg).

Biological activity of crude extract and pure compound

Duration of biological activity of crude extract (for Kanhangad and 56-2) and pure compound was evaluated in field. Four traps each containing 100 mg of the extract (crude/pure) were kept 3 m apart in weevil infested sweet potato fields and the number of weevils trapped was recorded on alternate days till the attraction of weevil was zero.

RESULTS

Laboratory evaluation of crude extract

Biological assay on ovipositional stimulant character of the crude extract (Kanhangad variety) showed that there was a positive response and the number of eggs laid by female weevils increased from 1.33 to 21.33 with increase in the amount of extract (2 to 100 mg) (Fig. 1).

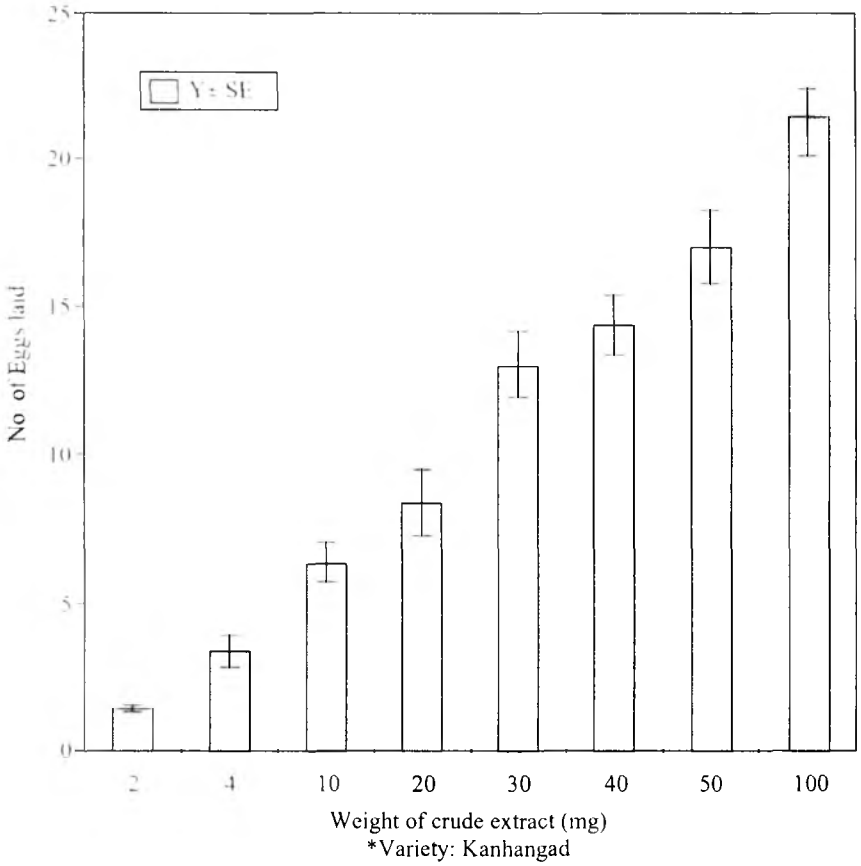

FIGURE 1. Efficacy of crude extract* as ovipositional stimulant.

Laboratory studies indicated that the crude dichloromethane extract of sweet potato periderm attracted both male and female weevils. The percentage of attraction increased with increase in the amount of the extract. With 25 mg of the crude extract the attraction was only 6.13 per cent but with 100 mg it increased to 42.45 with male:female ratio 3 : 1 (Table 1).

Field evaluation of crude extract

Field evaluation for the attraction of sweet potato weevil showed that 25 mg of the crude extract (Kanhangad variety) was not effective in attracting weevils. But with 50 mg of crude extract, the total number of weevils attracted was 21.5 and at 100 mg dose it was 71.5. Studies carried out with crude extracts from other varieties also showed that total number of weevils attracted varied with varieties. With RS-III-2 variety the number of weevils attracted was 22.75 and 83 respectively in the 50 and

TABLE 1. Effect of crude extract* on attraction of adult weevils at different levels in laboratory

Weight of the extract (mg)	No. of adult weevils released		No. of adult weevils trapped			Percentage of attraction	Male : Female ratio
	Male	Female	Male	Female	Total		
25	100	100	9.25 ± 0.74	3.00 ± 0.35	12.25 ± 1.08	6.13 ± 0.80	3.08 : 1
50	100	100	20.75 ± 2.40	6.50 ± 0.67	27.25 ± 2.85	13.63 ± 1.40	3.19 : 1
100	100	100	63.75 ± 2.61	21.15 ± 2.15	84.9 ± 3.51	42.45 ± 0.88	3.01 : 1

Values are Mean ± SE of 4 replications.
* Variety Kanhangad.

TABLE 2. Field studies on the effect of crude extract on attraction of adult weevils

Variety	Weight of the extract (mg)	No. of weevils trapped			Male:Female Ratio
		Male	Female	Total	
Kanhangad	25	0	0	0	0
	50	16.75 ± 1.19	4.75 ± 0.73	21.50 ± 1.92	3.53 : 1
	100	53.00 ± 3.70	18.50 ± 2.01	71.50 ± 4.75	2.86 : 1
RS-III-2	25	0	0	0	0
	50	19.50 ± 0.83	3.25 ± 0.49	22.75 ± 0.74	6.00 : 1
	100	64.00 ± 2.31	19.00 ± 2.49	83.00 ± 2.01	3.37 : 1
Sree Bhadra	25	0	0	0	0
	50	16.25 ± 1.25	5.00 ± 0.63	21.50 ± 0.92	3.25 : 1
	100	40.25 ± 2.24	12.50 ± 0.87	52.75 ± 1.71	3.22 : 1
56-2	25	—	—	—	—
	50	—	—	—	—
	100	49.25 ± 3.78	13.00 ± 1.40	62.25 ± 2.42	3.79 : 1

Values are Mean ± SE of 4 replications.

100 mg dose. In Sree Bhadra variety the number of weevils trapped was 21.50 and 52.75 in 50 and 100 mg dose respectively. In 56-2 variety 62.25 weevils got attracted at 100 mg dose. The male:female ratio among these varieties and different doses ranged from 2.86 to 6 : 1 (Table 2).

Purification of crude extract

Seven different fractions obtained during purification were tested for attraction of weevil. The fractions obtained from hexane (100%), hexane:ethyl acetate at 98 : 2, 94 : 6 and 80 : 20 did not show any attraction, while there was attraction in hexane:ethyl acetate mixture at 96 : 4 and 92 : 8 (26.6 and 17.4 per cent respectively). Maximum attraction (44.8%) was found in 90 : 10 combination.

TLC studies of the fractions obtained from 96 : 4 and 92 : 8 combinations

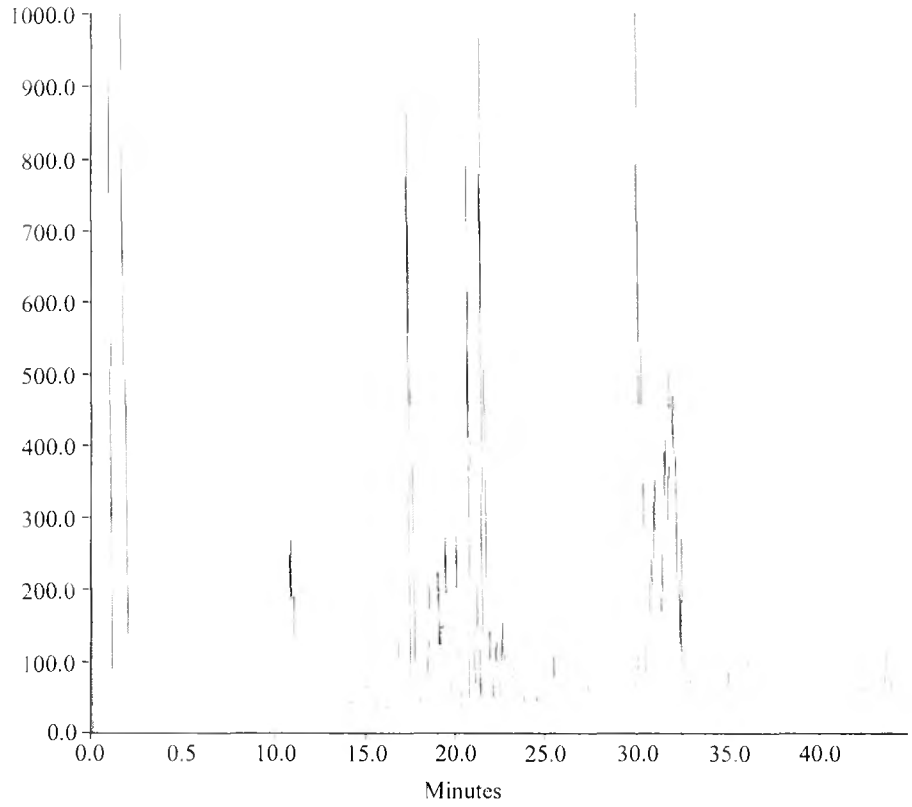

FIGURE 2. Gas chromatographic profile of crude dichloromethane extract of sweet potato periderm.

showed more than one spot. The fraction from 90 : 10, which gave maximum attraction, showed one single spot on TLC. This compound was further purified by recrystallisation from hexane.One gram crude extract of Kanhangad variety gave 45 mg of the pure compound and in Sree Bhadra it was 36.3 mg.

Gas chromatographic profile of crude extract showed five major peaks with retention time of 10.97, 17.67, 21.08, 21.87 and 30.43 min (Fig. 2). Recrystallised pure compound obtained from the 90 : 10 fraction gave a single peak with a retention time of 30.43 min (Fig. 3). The IR Spectrum of pure compound gave strong absorption band at 2960 cm^{-1}, medium absorption bands at 1700, 1400 and 1380 cm^{-1} and a broad band at 1090 cm^{-1}.

Efficacy of pure compound on attraction of weevil

Results obtained from the Laboratory evaluation with varying amounts of pure compound showed an increase in the attraction with increase in the dose. At 25 mg

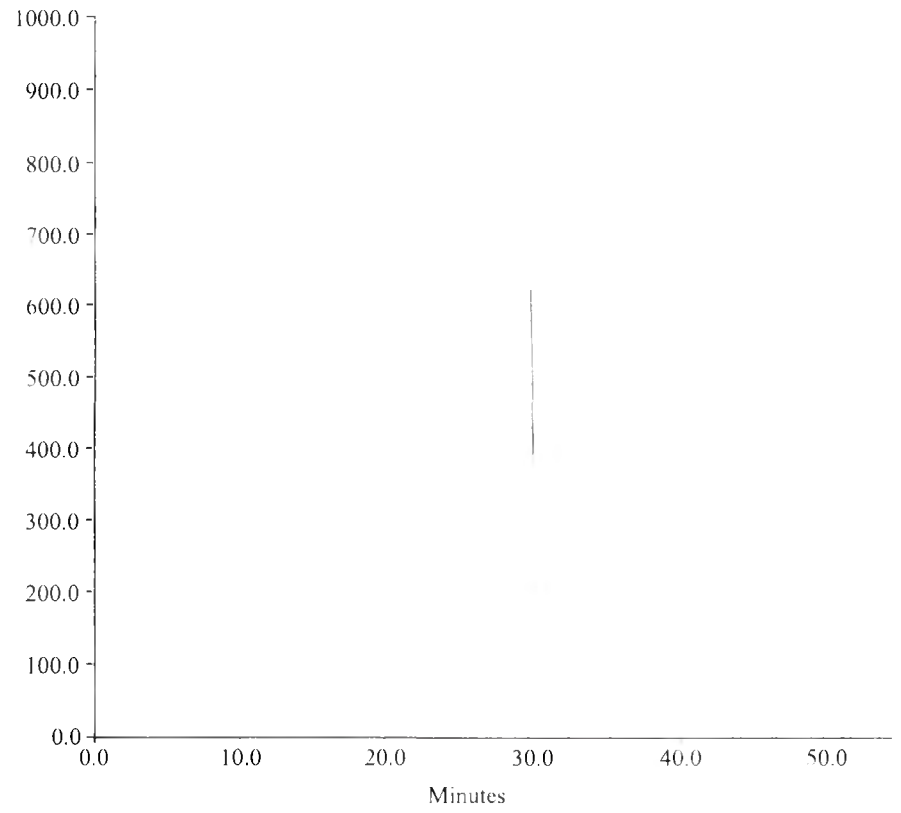

FIGURE 3. Gas chromatographic profile of pure compound.

the percentage of attraction was 6.5 and at 50 and 100 mg dose it was 11.6 and 60.88 respectively. The ratio of male:female attracted ranged from 2.06 to 2.90 : 1 (Table 3).

Field experiments for the attraction of weevils under natural conditions showed an increasing trend with the dose. At 25 mg pure compound, weevils were not attracted. On increasing the dose to 50 mg, the number of weevils attracted was 22.5 and with 100 mg the number was 80.75. The male:female ratio ranged from 3.50 to 3.82 : 1 (Table 4).

Biological activity of crude and pure compound

Studies carried out to know the life of crude extract from varieties Kanhangad, 56-2 and the pure compound under field conditions showed only a slight difference in the duration of biological activity of crude and pure compound. In both the cases the effect lasted for 18–22 days. The number of weevils attracted was more during the first 10 days with a sex ratio of 2.1 to 4.9 : 1 beyond which there was marked decline in the attraction for crude extract. The total number of weevils attracted during the 16th day

TABLE 3. Laboratory evaluation on kairomonal activity of pure compound on SPW

Weight of the compound (mg)	No. of weevils released		No. of weevils trapped			Percentage of attraction	Male female ratio
	Male	Female	Male	Female	Total		
25	100	100	8.75 ± 0.74	4.25 ± 0.54	13.00 ± 1.22	6.50 ± 0.61	2.06 : 1
50	100	100	21.75 ± 2.15	7.50 ± 1.25	29.25 ± 0.81	14.63 ± 0.80	2.90 : 1
100	100	100	82.75 ± 1.63	39.00 ± 2.06	121.75 ± 3.62	60.88 ± 1.81	2.12 : 1

Values are Mean ± SE of 4 replications.

TABLE 4. Field evaluation on kairomonal activity of pure compound on SPW

Weight of the compound (mg)	No. weevils trapped			Male:Female
	Male	Female	Total	
25	0	0	0	0
50	17.50 ± 1.14	5.00 ± 0.94	22.50 ± 1.09	3.50 : 1
100	64.00 ± 4.86	16.50 ± 2.48	80.75 ± 7.08	3.82 : 1

Values are Mean ± SE of 4 replications.

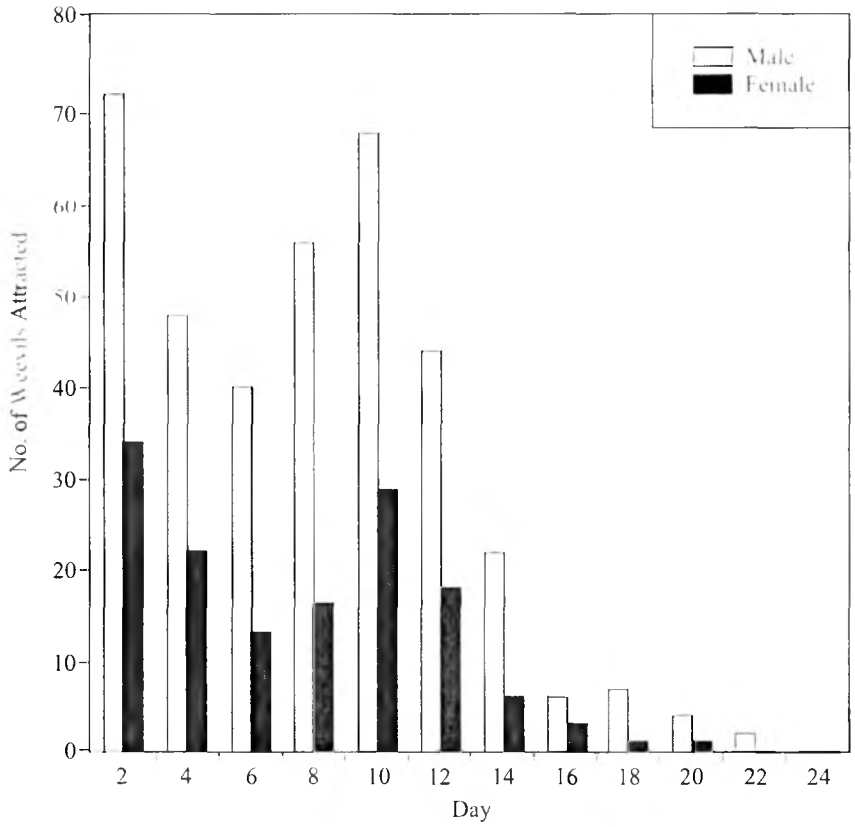

FIGURE 4. Kairomonal activity of crude extract (100 mg) in field variety Kanhangad.

of the experiment was less than 10 (Figs 4 and 5). But in case of the pure compound during the 16th day the number of weevils attracted was 50 (Fig. 6). During the last day only male weevils were attracted. The male:female ratio during the period ranged from 2 to 7 : 1.

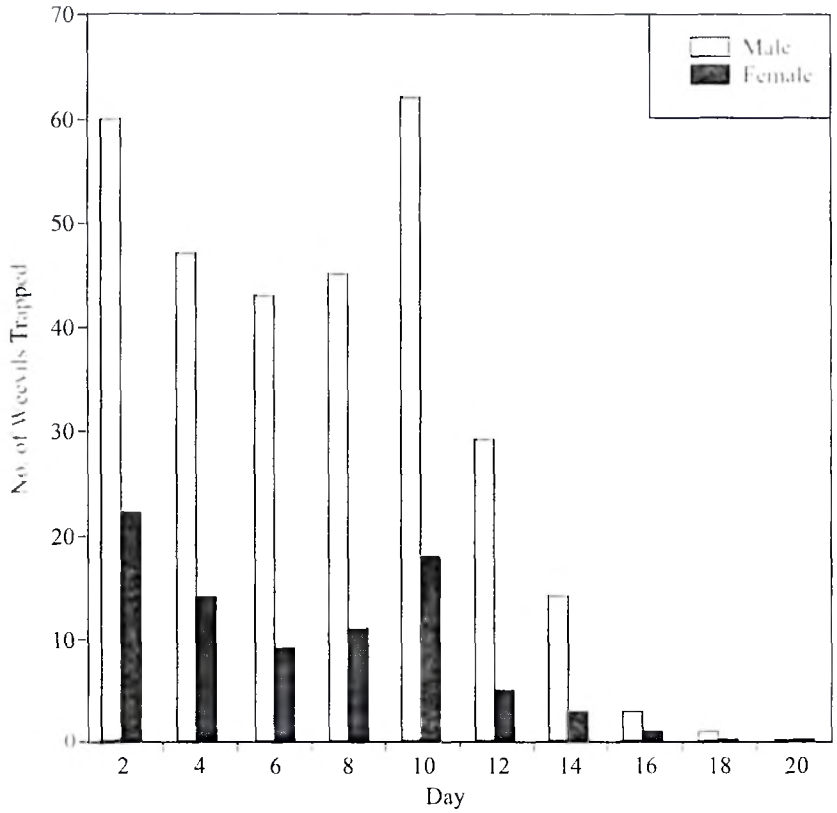

FIGURE 5. Kairomonal activity of crude extract (100 mg) in field variety 56-2.

DISCUSSION

Presence of a behaviour modifying semiochemical in sweet potato tubers especially for attraction of adult weevils was reported by Palaniswami and Mohandas (1994). The ovipositional stimulant nature in sweet potato periderm was indicated when the crude extract of periderm induced egg laying and showed an increase in the number of eggs laid by weevil with increase in concentration of the extract. Both laboratory and field studies on attraction of adult weevils indicated the potentiality of the compound as an attractant to both males and females. Therefore this compound was found to act as an attractant as well as an ovipositional stimulant. Wilson *et al.* (1989, 1990, 1991) reported the sweet potato periderm extract as an ovipositional stimulant. In our studies among the four varieties tested, RS-III-2 revealed maximum attraction of both males and females followed by other varieties. This implied that the relative percentage of the compound attracting both male and female weevils was higher in RS-III-2. The variation in the yield of pure compound from one gram of crude extract from two varieties indicated that the compound is present at varying levels in different

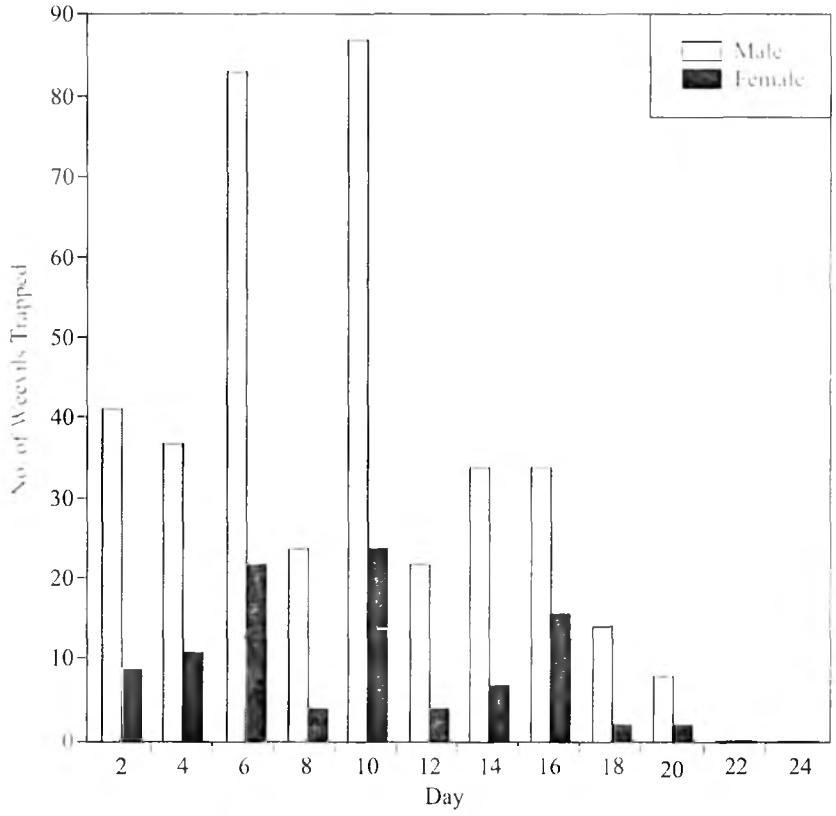

FIGURE 6. Kairomonal activity of pure compound (100 mg) in field.

varieties. However it warrants further investigation. Nottingham *et al.* (1987) reported that leaves and storage roots of sweet potato were attractive to female weevils but the response varied with cultivars used. However attraction of males to the compound is a new information.

IR spectral analysis of the compound confirmed that the compound is acetate of boehmerol, a pentacyclic triterpenoid and it is in conformity with Wilson *et al.* (1989). Retention time obtained in Gas Chromatographic analysis of the compound at 30.43 min is comparable with the reports of Son *et al.* (1990).

Biological activity of crude extract and pure compound is more or less on par. In both cases biological activity lasted for 18–22 days, indicating boehmeryl acetate is not affected by the presence of other compounds in the sweet potato periderm. Present investigations revealed the potentiality of boehmeryl acetate/sweet potato periderm extract as an ovipositional stimulant/weevil attractant at 100 mg dose. Camacho *et al.* (1998) reported that of subalpine fir compounds acted as kairopmone to both sexes of western balsam bark beetle (*Dryocoetes confusus* Sw.). There is lot of scope for integrating this behaviour modifying kairomone in IPM of *C. formicarius*.

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REFERENCES

Agelopoulos, N., Birkett, M. A., Hick, A. J., Hooper, A. M., Pickett, J. A., Pow, E. M., Smart, L. E., Smiley, D. W. M., Wadhams, L. J. and Woodcock, C. M. (1999) Exploiting semiochemicals in insect control, *Pestici. Sci.* **55(3)**: 225–235.

Camacho, A. D., Pierce, H. D. Jr. and Borden, J. H. (1998) Host compounds as kairomones for the western balsam bark beetle *Dryocoetes confusus* Sw. (Coleoptera: Scolytidae), *J. App. Entomo.* **122(6)**: 287–293.

Hall, D. R. (1998) *Inaugural Lecture Series. ISBN 1 86166-070-7 Natural Resources Institute*, University of Greenwich: U.K, 42.

Nottingham, S. F., Wilson, D. D., Severson, R. F. and Kays, S. J. (1987) Feeding and oviposition preferences of the sweet potato weevil *Cylas formicarius elegantulus* (Summers), on the outer periderm and exposed inner core of selected sweet potato cultivars, *Entomol. Exp. Appl.* **45**: 271–275.

Palaniswami, M. S. (1994) Biological control of pests of tuber crops. In: *Advances in Horticulture. Vol. 8. Tuber Crops*, Chadha, K. L. and Nayar, G. G.: (Editors). MPH, New Delhi, 513–522.

Palaniswami, M. S. and Mohandas, N. (1994) Studies on attractants of adult weevils of *Cylas formicarius* F., *J. Root Crops.* **20(1)**: 44–52.

Palaniswami, M. S., Mohandas, N. and Saraswathy, P. (1991) Onfarm survey on the incidence, extent and intensity of *Cylas formicarius* F. damage on sweet potato in Kerala, *J. Root Crops* **17(special)**: 180–196.

Sorenson, K. A. (1984) Impact of the sweet potato weevil in the South-East, *Proceedings of Sweet Potato Weevil Workshop*, Mullen, M. A. and Sorenson, K. A.: (Editors). N.C. State University: Raleigh, 1–16.

Son, K. C., Severson, R. F., Arrendale, R. F. and Kays, S. J. (1990) Isolation and characterization of pentacyclic triterpene ovipositional stimulant for the sweet potato weevil from *Ipomoea batatas* (L.) Lam, *J. Agri. Food Chem.* **38**: 134–137.

Wilson, D. D., Son, K. C., Nottingham, S. F., Severson, R. F. and Kays, S. J. (1989) Characterisation of ovipositional stimulant from the surface of sweet potato weevil *Cylas formicarius elegantulus* (Summers), *Entomol. Exp. Appl.* **51**: 71–75.

Wilson, D. D., Son, K. C., Severson, R. F. and Kays, S. J. (1990) Effect of pentacyclic triterpene from sweet potato storage roots on oviposition by the sweet potato weevil (Coleoptera: Curculionidae), *Environ. Entomol.* **19**: 1663–1665.

Wilson, D. D., Severson, R. F. and Kays, S. J. (1991) Ovipositional stimulant of *Cylas formicarius* in sweet potato: Isolation, identification and development of analytical screening method, In: *Sweet Potato Pest Management—A Global Perspective*, Jansson, R. K. and Raman, K. V.: (Editors). Westview Press: USA, 221–233.

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The Intrinsic Rate of Natural Increase of a Harpactorine Reduviid *Rhynocoris kumarii* Ambrose and Livingstone on Three Lepidopteran Insect Pests

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ABSTRACT The biology, life tables and intrinsic rate of natural increase of a harpactorine reduviid *Rhynocoris kumarii* Ambrose and Livingstone on three lepidopteran insect pests *viz.*, *Spodoptera litura* Fab., *Earias vittella* Fab. and *Corcyra cephalonica* Stainton have been constructed under laboratory conditions. Maximum longevity and fecundity and minimum total developmental period have been observed in *R. kumarii* by rearing on the larvae of *S. litura*. The net reproductive rate (R_0) highest on *S. litura* (83.80) followed by *E. vittella* (66.70) and *C. cephalonica* (56.90). The study reveals that the intrinsic rate of natural increase (r_m) and finite rate of increase (λ) have been 0.050 and 1.051 for *S. litura* 0.042 and 1.043 for *E. vittella* and 0.039 and 1.039 females/female/day for *C. cephalonica*. *Spodoptera litura* has been found to be the most suitable prey species for laboratory multiplication of *R. kumarii*. © 2000 Association for Advancement of Entomology

KEYWORDS: Reduviid, *Rhynocoris kumarii*, development, life table, insect pests.

INTRODUCTION

Reduviids have been documented as important natural enemies, suppressing several pests, especially lepidopteran insect pests (Ambrose, 1996). Conservation and augmentation of natural enemies, their mass rearing and release at appropriate stage and condition are the major components in Integrated Pest Management (IPM). The quality and quantity of nutrients of the prey influence not only the growth rate and survival of the predator but also the fecundity and life table characteristics such as generation time as well as intrinsic rate of population increase (George *et al.*, 1998). The prey insects significantly influence vital phenomena such as rate of development, survival and reproductive potential of insects which ultimately determine the rate of population build up. Hence life table studies of predators on different insect pests are of paramount importance in assessing the innate capacity for population build up and informations on population dynamics enable efforts on augmentation and subsequent release of

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the predator in the field for the control of insect pests. Life table studies on predatory insects are rare. The contributions of Jones *et al.* (1988), Makhmoor and Verma (1989), Bellows *et al.* (1992) Singh and Singh (1994), Sharma *et al.* (1994), Kumar and Velusamy (1995), Sharma and Bhalla (1995), Gupta and Singh (1996), Venkatesan *et al.* (1997) and George *et al.* (1998) have exposed several facets in this area of research. A comparative study on the life tables of a harpactorine reduviid *Rhynocoris kumarii* Ambrose and Livingstone on three important lepidopteran insect pests *viz.*, *Spodoptera litura* Fab., *Earias vittella* Fab. and *Corcyra cephalonica* Stainton has been attempted. *R. kumarii* has been recorded as an efficient predator on various economically important crops (Ambrose, 1999).

MATERIALS AND METHODS

Adults of *R. kumarii* were collected from Marunthuvazhmalai scrub jungle (altitude 50.0 ± 5.77 mts; latitude 77°35 E and 8°14′ N), Kanyakumari district of Tamil Nadu, South India and reared in the laboratory separately on mature fourth instar larvae of *S. litura, E. vittella* and *C. cephalonica* in plastic containers (6 cm diameter, 6 cm height) covered on top by perforated plastic lids. A cohort consisting of 100 eggs of the predator from each such reared set has been used to construct life tables. These eggs were allowed to hatch in small plastic vials (4 cm diameter, 4 cm height) in which moistened cotton swabs were kept for maintaining optimum humidity (85%). The cotton swabs were changed periodically to prevent fungal attack. After noting egg hatchability and incubation period all the newly hatched nymphs were reared individually in plastic vials of similar dimensions. Fourth instar larvae of *S. litura, E. vittella* or *C. cephalonica* were provided as prey for the respective cohort. Observations were recorded. They include stadial period, number of successfully emerged adults, their total period of development, age specific mortality in the various developmental stages including adult period and egg production rate of mated females in their life time. Life tables were constructed according to the methods recommended by Birch (1948) and elaborated by Howe (1953), Watson (1964), Laughlin (1965) and Southwood (1978).

In life table statistics, the intrinsic rate of increase has been determined by using the equation $\Sigma e^{-r_m \times l_x m_x - 1}$.

Where e is the base of natural logarithms, x is the age of the individuals in days, l_x is the number of individuals alive at age x as the proportion of 1, and m_x is the number of female offsprings produced per female in the age interval x. The sum of products $l_x m_x$ is the net reproductive rate (R_o). The rate of multiplication of population for each generation has been measured in terms of females produced per generation. The precise value of cohort generation has been calculated as follows.

$$T_c = \frac{\Sigma l_x m_x}{R_o}.$$

The arbitrary value of innate capacity for increase r_c has been calculated applying the

TABLE 1. Biology of *R. kumarii* on three insect pests ($x \pm$ SD)

Parameters	Prey species		
	S. litura	*E. vittella*	*C. cephalonica*
Incubation period	8.74 ± 0.83^a	8.91 ± 0.98^a	9.41 ± 0.86^b
Total developmental period	63.45 ± 3.18^a	70.43 ± 2.96^b	74.12 ± 3.14^b
Preoviposition period	17.43 ± 1.21^a	19.88 ± 2.32^{ab}	21.36 ± 2.19^b
Longevity	111.46 ± 5.34^a	94.16 ± 6.74^b	83.33 ± 5.99^c
Fecundity	221.46 ± 14.32^a	207.18 ± 11.49^a	189.32 ± 9.41^c

Values followed by similar superscripts 'across a row' are not statistically different at 0.05 level by Tukey test.

equation

$$r_c = \frac{\log_e R_o}{T_c}$$

This is an appropriate r_m value. The values of negative exponent of e^{-rm} ascertained form this experiment often lay outside the range. For this reason both sides of the equation have been multiplied by a factor of $\Sigma e^{-7(x+0.5)r_m} l_x m_x - 1096.6$ (Birch, 1948 and Watson, 1964). The two values of $\Sigma e^{-7(x+0.5)r_m} l_x m_x$ have been then plotted on the horizontal axis against their respective arbitrary r_m on the vertical axis. Two points have been then joined to give a line which is intersected by vertical line drawn from the desired value of $e^{-7(x+0.5)r_m} l_x m_x$ (1096.6).

The point of intersection gives the value of r_m accurate to three decimal places. The precise generation time (T) has been then calculated from equation

$$T = \frac{\log_e R_o}{r_m}$$

The finite rate of increase (λ) has been calculated as e^{r_m}. This λ represents the number of individuals added to the population per female per day (Siddiqui *et al.*, 1973). The weekly multiplication of predator population has been calculated as $(e^{r_m})^7$. The doubling time has been further calculated as $\log 2 / \log \lambda$.

RESULTS AND DISCUSSION

The incubation period of *R. kumarii* on *S. litura* is 8.74 ± 0.83 days and it extends to 8.91 ± 0.98 and 9.41 ± 0.86 days on *E. vittella* and *C. cephalonica*, respectively. The total developmental period on *S. litura* is 63.45 ± 3.18 days an it extends to 70.43 ± 2.96 and 74.12 ± 3.14 days respectively on *E. vittella* and *C. cephalonica*. Increased adult longevity is evident in *R. kumarii*, reared on *S. litura* (111.84 ± 5.34 days) than on *E. vittella* (94.16 ± 6.74 days) and on *C. cephalonica* (83.33 ± 5.99 days). Similarly, maximum number of eggs per female has been also observed in *R. kumarii*, reared on *S. litura* (221.46 ± 14.42). The egg number decreases to 207.18 ± 11.49 and 189.32 ± 9.41 when reared on *E. vittella* and *C. cephalonica*, respectively (Table 1).

The net reproductive potential (R_o) of *R. kumarii* on *S. litura* has been worked

TABLE 2. Population growth parameters of *R. kumarii* on three insect pest

Parameters	Insect pests		
	S. litura	*E. vittella*	*C. cephalonica*
Gross reproductive rate	112.00	105.00	97.00
Net reproductive rate (R_0)	83.80	66.70	56.90
Mean length of generation (T_c)	97.28	110.61	111.79
Estimated value of intrinsic rate of increase in numbers (r_c)	0.045	0.038	0.036
Corrected r_m	0.050	0.042	0.039
True generation time (T)	88.57	100.01	103.62
Finite rate of increase in numbers (λ)	1.051	1.043	1.039
Doubling Time	13.87	16.45	18.13
Weekly multiplication rate	1.419	1.342	1.314
Annual rate of increase	8.4×10^7	4.5×10^6	1.5×10^6
Hypothetical female in F_2 generation $(R_0)^2$	7022.44	4448.89	3237.61

out as 83.80, with a mean length of generation (T_c) 97.28 days. Decreased net reproductive rate (66.70 and 56.90) and increased mean length of generation (110.61 and 111.79 days) were recorded for *E. vittella* and *C. cephalonica* respectively. The true intrinsic rates of natural increase (r_m) and the finite rate of increase of *R. kumarii* are 0.050 and 1.051, 0.042 and 1.043 and 0.039 and 1,039 females/female/day on *S. litura*, *E. vittella* and *C. cephalonica* respectively. However, these rates of increase are attainable only under favourable conditions when sufficient supply of the respective prey larvae are provided. At this rate, *R. kumarii* is capable of multiplying 1.419, 1.342 and 1.314 times per week and 8.4×10^7, 4.5×10^6 and 1.5×10^6 times per annum under prevailing (optimum) temperature and humidity (Table 2).

The shortest total developmental period recorded in *R. kumarii* when reared on *S. litura* might be the consequence of better nutrition obtained by the predator with minimum expenditure of energy during predation on less number of prey individuals of larger size and more body tissue. In a broad sense, this observation agrees with the concept of Slansky (1982) which establishes a correlation between prey preference and accelerated developmental rate of predators. Similar observations have been reported earlier by Anderson (1962) in *Anthocoris* spp., De Clereq and Degheelee (1994) on *Podisus maculiventris*, Venkatesan *et al.* (1997) on a reduviid *Cydnocoris gilvus* and George *et al.* (1998) on reduviid *Acanthaspis siva*.

The highest fecundity and maximum longevity of adult females when fed on the larve of *S. litura* has been noted in this investigation. It also appears to be a reflection of the superior nutritional quality of such prey as diet. This conclusion concurs with the inference drawn by Venkatesan *et al.* (1997) and George *et al.* (1998). They also observed high egg output and maximum longevity in reduviids *C. gilvus* and *A. siva* when reared on *S. litura*, amongst three types of tested prey feed.

The fact that in the present study, larve of *S. litura* as prey food of *R. kumarii* has been found to facour (a) faster development, (b) prolonged longevity, (c) higher

fecundity, (d) higher net reproductive rate and (e) shorter population doubling time of the predator, clearly serving as a pointer to suggest that, in biocontrol programmes involving insect predators and their prey in pest management practices, considerable attention has to be paid to select appropriate prey species for the population build-up of preators.

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REFERENCES

Ambrose, D. P. (1996) Assassin bugs (Insects: Heteroptera: Reduviidae) in biocontrol: Success and strategies, a review, In: *Biological and Cultural Control of Insect Pests, an Indian Scenario*, Ambrose, D. P.: (Editor) Adeline publishers: Tirunelveli, 262–284.

Ambrose, D. P. (1999) *Assassin Bugs*, Oxford and IBH: New Delhi, 5 pp.

Anderson, N. H. (1962) Growth and fecundity of *Anthocoris* spp. reared on various prey (Heteroptera: Anthocoridae), *Entomol. Exp. Appl.* **5**: 40–52.

Bellows, T. S. Jr., Van Driesche, R. G. and Elkinton, J. S. (1992) Life table construction and analysis in the evaluation of natural enemies, *Ann. Rev. Entomol.* **37**: 587–614.

Birch, S. (1948) The intrinsic rate of natural increase in an insect population, *J. Anim. Ecol.* **17**: 15–26.

De, Clercq P. and Degheelee (1994) Laboratory measurement of predation of *Podisus maculiventris* and *P. sagitta* (Hemiptera: Pentatomidae) on beet armyworm (Lepidoptera: Noctuidae), *J. Econ. Entomol.* **87(1)**: 6–8.

George, P. J. E., Seenivasagan, R. and Kannan, S. (1998) Influence of prey species on the development and reproduction of *Acanthaspis siva* Distant (Heteroptera: Reduviidae), *Entomon* **23(4)**: 69–75.

Gupta, P. R. and Singh, I. (1996) Predation preference and reproductive performance of the San Jose scale, In: *Biological and Cultural Control of Insect Pests, an Indian Scenario*, Ambrose, D. P.: (Editor) Adeline publishers: Tirunelveli, 289–296.

Howe, R. W. (1953) The rapid determination of intrinsic rate of increase of an insect population, *Ann. Appl. Biol.* **40**: 134–155.

Jones, R. W., Gilstrap, F. E. and Andrews, K. L. (1988) Biology and life tables for the predaceous earwig, *Doru taeniatum* (Derm: Forficulidae), *Ent. Soc. Am.* **33**: 47–54.

Kumar, M. G. and Velusamy, R. (1995) Life tables and intrinsic rates of increase of *Cyrtorhinus lividipennis* Reuter (Heteroptera: Miridae), *J. Biol. Control* **9**: 82–84.

Laughlin, R. (1965) Capacity for increase: A useful population statistics, *J. Anim. Ecol.* **34**: 77–91.

Makhmoor, H. D. and Verma, A. K. (1989) The intrinsic rate of natural increase of *Metasyrphus confrater* (Wied). (Diptera: Syrphidae) a predator of the cabbage aphid (Homoptera: Aphididae) *Proc. Indian Nat. Acad. Sci.* **B55**: 79–84.

Sharma, K. C. and Bhalla, O. P. (1995) Life table studies on *Eupeodes confrater* (Fabricius) (Diptera: Syrphidae) a predator of the cabbage aphid *Brevicoryne brassicae* (Linnaeus) (Homoptera: Aphididae), *J. Biol. Control* **9**: 78–81.

Sharma, K. C., Bhalla, O. P. and Chauhan, U. (1994) Life table and intrinsic rate of increase of *Eupedes frequens* Matsumura a predator of *Brevicoryne brassicae* (Aphididae: Homoptera) infesting cauliflower, *J. Biol. Control* **8(1)**: 56–58.

Siddiqui, W. H., Bariow, C. A. and Randolph, P. A. (1973) Effect of some constant and alternating temperatures on population growth of the pea aphid *Acyrthosiphon pisum* (Homoptera: Aphididae), *Can. Ent.* **105**: 145–146.

Singh, H. S. and Singh, R. (1994) Life fecundity table of *Coccinella septumpunctata* Linn. predating on mustard aphid (*Lipaphis erysimi* Kalt) under laboratory and field conditions, *J. Ent. Res.* **18**: 297–303.

Slansky (1982) Insect nutrition: An adoptionists perspective, *Flo. Ent.* **65**: 47–71.

Southwood, T. R. E. (1978) *Ecological Methods: With Particular Reference to the Study of Insect Population*, Chapman and Hall: London, 524 pp.

Venkatesan, S., Seenivasagan, R. and Karuppasamy, G. (1997) Influence of prey species on feeding response, development and reproduction of the reduviid *Cydnocoris gilvus* Burm (Reduviidae: Heteroptera), *Entomon* **22(1)**: 21–27.

Watson, T. F. (1964) Influence of host plant condition on population increase of *Tetranychus telerium* (L.). (Acarina: Tetranychidae), *Hilgardia* **35**: 273–322.

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A New Species of Discolomidae from Mizoram, India (Coleoptera: Cucujoidea)

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ABSTRACT. A new discolomid species, *Aphanocephalus maculipennis*, is described from the northeast Indian state of Mizoram, bringing the total number of Indian species of the genus to 5. A key to the Indian species of *Aphanocephalus* Wollaston is included. 2000 Association for Advancement of Entomology

KEYWORDS: Coleoptera, Discolomidae, *Aphanocephalus*, Wollaston, new species, Mizoram.

INTRODUCTION

The Discolomidae, otherwise referred to as Notiophygidae, are a moderately large family of cucujoid beetles comprising about 400 species under 18 genera. The discolomids are small (1–5 mm), rounded beetles with glabrous or setose surface, deeply inserted head into prothorax, 8- to 10-segmented antenna with single segmented club, hind coxae largely concealed and appear globular, and trimerous tarsi. The family occur in tropical and subtropical parts of both Old and New Worlds with more diverse forms in the eastern hemisphere. Pal (1992) recorded this family from the Indian state of Arunachal Pradesh, and described three more species from Sikkim bringing the Indian species of the family to five (Pal, 1996). Four of these species belong to *Aphanocephalus* Wollaston and one to *Paramaschema* Heller. During field work in the Lushai Hills of Mizoram state, the beetles of this family were noticed in the woodlands of Champhai area, very close to the Myanmar border indicating the possibility of occurrence of this primarily wood inhabiting forms in humid forest areas in the neighbouring country. This discolomid species of *Aphanocephalus* is strikingly different from all known Indian species. This new species is described in this paper.

Genus *Aphanocephalus* Wollaston.

Aphanocephalus Wollaston, 1913, *Ent. mon. Mag.* **9**: 278 (Type: *hemisphaericus* Wollaston).

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Diagnosis

Broadly ovoid body, strongly convex dorsally and flattened ventrally, glabrous vestiture consisting of minute setae not generally visible at lower magnification; transverse head with small eyes, 9-segmented antenna with a large club; strongly transverse prothorax with weakly rounded and smooth lateral sides; front coxal cavities closed behind, mesocoxal cavities closed and sternal fitting in a straight line, hind coxae widely separated; abdomen with broad intercoxal process and freely articulated ventrites.

Aphanocephalus maculipennis sp. nov. (Figs 1–3)

General appearance (Fig. 1) oblong, ovate, about 1.28 times as long as broad, convex dorsally and flattened ventrally, shiny, finely punctate-pubescent; dark brown except paler front and sides of pronotum, border and two ovoid spots of elytra; antenna paler than dorsum.

Head small, exposed part distinctly transverse, almost concealed from above by pronotum, fronto-clypeal suture situated just in front of antennal insertions, clypeus with pubescence denser and more distinct than on pronotum; eyes small; partly visible from dorsal side; antenna (see Fig. 2) short, shorter than pronotum, scape moderately large, pedicel narrower and a little elongatge, segments 3–5 more or less elongate, segments 6–7 short and subequal, segment 8 wider than 7, club large, elongate, little more than one-third as long as antenna and with single preapical annulation.

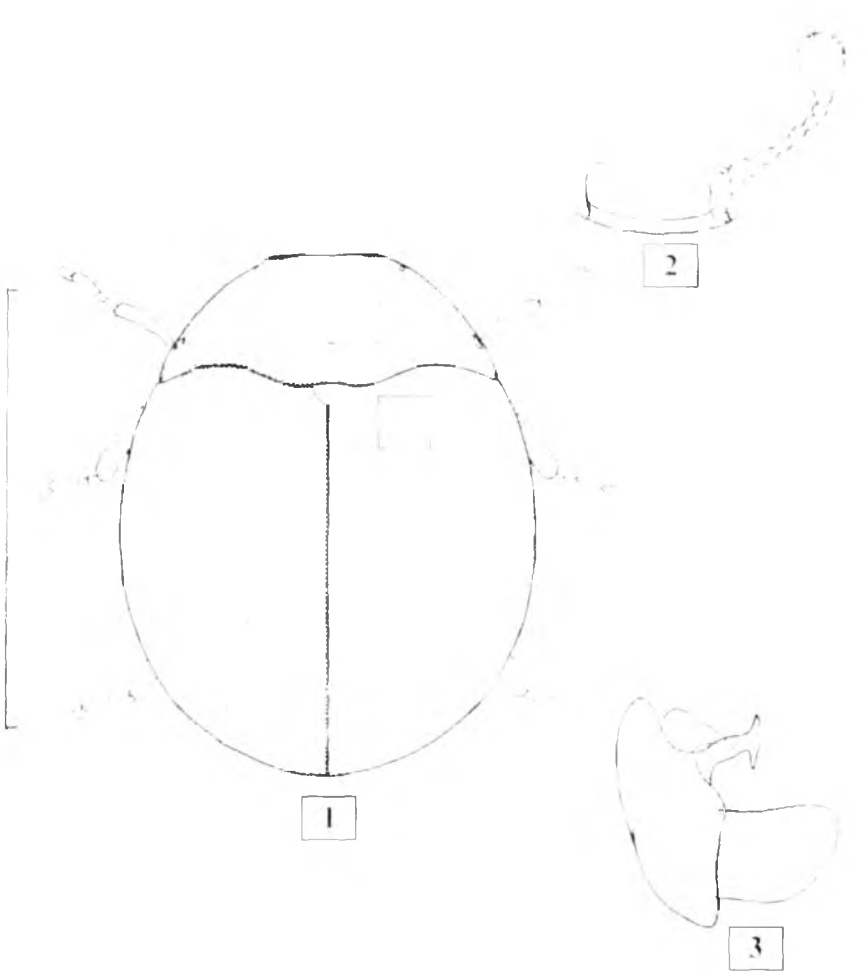
Prothorax strongly transverse (2.8 : 1.00); narrowed in front, front margin slightly emarginate, front angles bluntly rounded, lateral margin feebly rounded and finely bordered, two lateral pits situated close to anterior angle and at posterior third, posterior angles acute, basal margin distinctly sinuate on either side of middle; sides and front margin or pronotum paler than remaining part; punctation fine and sparse, pubescence very short, scanty.

Elytra about as broad as long, more than twice (2.58 : 1.0) as long as prothorax, basal margin emarginate and fitting closely with prothorax, humeral angles blunt, widest near middle; sides evenly rounded, finely bordered and a little explanate; a pair of admedian, ovoid, paler spots near middle, edges of which are moderately well defined; punctures simple, point-like impressions on cuticle, sparse, pubescence short and scanty.

Ventral side paler, prosternum indistinctly punctate and finely pubescent; metasternum with fine dense punctures, little weaker mesally, punctation on abdominal ventrites almost similar to that of metasternum; legs yellowish-brown with apical third of femora and tibiae lighter; epipleura flat, broad basally and acute for entire length.

Aedeagus (Fig. 3) on one side, median lobe forming a tube with distal end broad and blunt; tegmen forming a cap-piece enveloping median lobe, its proximal end with an elongate projection, the tip of which is broadened.

Measurements of holotype: Total length 1.54 mm., length of antenna 0.42 mm., length and width of prothorax 0.48 mm. and 1.00 mm., length and width of elytra 1.24 mm. and 1.20 mm.



FIGURES 1–3. *Aphanocephalus maculipennis* sp. nov. 1, Dorsal view (scale = 1.0 mm.); 2, Exposed part of head and antenna (scale = 0.5 mm.); 3, Aedeagus, Lateral view.

Holotype: India: Mizoram, Aizawl dist., Ruantlang, 1325 m., 10 km. 0-Champhai, 1.ii.1995, T. K. Pal, *ex. Pinus kesiya* (beneath bark).

Paratypes 9 ex., same data as holotype (Zoological Survey of India).

REMARKS

This species differs from other Indian species of *Aphanocephalus* in having a pale hemispherical spot near middle of each elytron (In all other Indian species elytra is devoid of paired hemispherical spots near middle, but only its sides are narrowly

paler). This species shows certain resemblances with the Australian species *A. sedlaceki* John in having similar elytral colour pattern and paler margin of pronotum, but *sedlaceki* is more globose in shape, with different structure of aedeagus. This species also resembles, to some extent, the Sikkimese species *A. convexus* Pal in facies and aedeagal features. But it differs from *convexus* by the proximal finger-like projection of tegmen widened at tip and shape of median lobe, apart from the dorsal colour pattern (In *convexus* proximal finger-like projection of tegmen is more or less uniformly broad with a rounded tip, median lobe foot-shaped distally with a little constriction above distal margin).

Etymology

The species name refers to the paired hemispherical pale spots on the dark brown elytra.

KEY TO THE SPECIES OF *APHANOCEPHALUS* WOLLASTON FROM INDIA

1. Front margin of prothorax rather deeply and widely emarginate, anterolateral margin of pronotum not paler than remaining part, one pair of lateral marginal pits of pronotum present near humeral angles and those near basal third absent; frontoclypeal suture situated considerably in front of antennal bases; aedeagus with proximal and distal edge of tegminal cap spatulate and beset with hairs ..... *superbus* Pal

   – Front margin of prothorax either unemerginate or slightly emarginate, anterolateral margin of pronotum paler (reddish-brown) than remaining part (blackish-brown), two pairs of lateral marginal pits near humeral angles and near basal third present; frontoclypeal suture situated just in front of antennal bases; aedeagus with proximal and distal ends of median lobe broad, distal edge of tegminal cap devoid of hairs 2

2. Antennal club segment with single preapical annulation; front margin of prothorax almost unemarginate or indistinctly emarginate 3

   – Antennal club segment with two preapical annulations; front margin of prothorax narrowly and slightly emarginate 4

3. Only sides of elytra narrowly paler, remaining part uniformly dark brown; aedeagus with median lobe foot-shaped distally, a little constricted above distal margin, proximal end of tegminal cap forming a finger-like projection with rounded tip ..... *convexus* Pal

   – A pair of admedian hemispherical paler spots on elytra in addition to marginal pale border; aedeagus with median lobe broadened below middle, slightly rounded distally, proximal end of tegminal cap with an elongate projection and tip of which broadened ..... *maculipennis* sp. nov.

4. Antero-lateral pale border of protonum uninterrupted and continuous throughout its length; aedeagus with distal end of median lobe narrowed and rather broadly pointed ..... *johni* Pal

– Antero-lateral border of pronotum interrupted near middle, along front margin; aedeagus with distal end of median lobe broad and blunt ..... *sikkima* Pal

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REFERENCES

Pal, T. K. (1992) Discolomidae, a beetle family new from India, *Hexapoda* **4(1)**: 45–56.
Pal, T. K. (1996) Three new species of Discolomidae (Coleoptera: Clavicornia) from Sikkim, India, *Hexapoda* **8(1)**: 9–17.

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Food Utilization, Growth and their Relative Rates in the Lines of Silkworm, *Bombyx mori* L. Selected for Pupal Weight

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ABSTRACT: The present study was undertaken to investigate the effect of selection of food utilization, growth and relative rates in high, medium, low and control lines after eight generations of selection for pupal weight in silkworm, *Bombyx mori* L. Food utilization and growth were found to increase with larval development in the selection and control lines. However within the lines, food utilization was higher in high and medium and lower in low lines. The relative rates of consumption, assimilation and metabolism decreased with the increase of age from first to fourth instar and increased sharply towards fifth instar in the selected and control lines of both NB18 and Pure Mysore. The selection for pupal weight in silkworms favoured the low selection lines for rates of consumption, assimilation and metabolism. The magnitude of growth rate was higher in high and medium lines of NB18 whereas only marginal difference in growth rate was noticed within the lines of Pure Mysore.
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KEYWORDS: Food utilization, growth, relative rates, pupal weight, *Bombyx mori*.

INTRODUCTION

Genetic variations in the body weight due to selection may alter the mechanisms which affect food utilization efficiencies as observed in mice (Fowler, 1962; Lang and Legates, 1969; Roberts, 1973) chicken (Pym and Nicholas, 1979; Siegel *et al.*, 1984) and *Tribolium castaneum* (Medrano and Gall, 1976). Nutritional studies in silkworm with respect to food utilization (Matsumura *et al.*, 1955; Ueda, 1982; Horie and Watanabe, 1983), relation between growth, body weight, food digested and injested, silk gland weight (Ueda, 1965; Ueda and Suzuki, 1967; Ueda *et al.*, 1971), food consumption and relative rates (Mathavan and Pandian, 1974; Mathavan *et al.*, 1987) have been elucidated. The interstrain differences in food efficiency have been studied in temperate (Yamamoto and Fujimaki, 1982) and tropical breeds (Periasami and

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Radhakrishnan, 1985; Benchamin and Jolly, 1984; Remaderi *et al.*, 1991; Remadevi *et al.*, 1992; Magadum *et al.*, 1966a,b). The purpose of this investigation was to characterise the correlated changes in food utilization, growth and relative rates in the selected lines of silkworm, *Bombyx mori* L.

MATERIALS AND METHODS

The study was undertaken with pupal weight as a trait under selection in three directions, i.e., high, medium, and low in two genetically divergent strains namely, a polyvoltine Pure Mysore (PM) and a bivoltine, NB18. A detailed description of the three selection lines have been given by Puttaraju and Rajanna (1997). After eight generations of selection, the investigation on food utilization and growth was carried out in high, medium, low and control lines. In each line, three replications of five hundred larvae up to third instar and 25 larvae from fourth instar to cocooning were used for this experiment. Silkworms were reared following standard procedure (Krishnaswami, 1978) and the larvae were fed *ad libitum* four times a days with K2 mulberry leaves. A parallel batch of silkworms was also maintained to replenish the missing larvae and also for determining dry weight values. Faecal pellets were collected separately from each replicate once during first and second instars, twice in third instar and daily during fourth and fifth instars. The collected faecal pellets were oven dried at 90 °C for weight constancy.

The scheme for energy budget followed here is the modified IBP formula (Petrusewicz and Macfadyen, 1970), $C = P + R + F$; where C the food consumed, P the growth, R the weight loss due to metabolism and F the undigested food defaecated including nitrogenous excretory products. Food consumed (C) or food ingested was estimated following the method of Mathavan and Pandian (1974). Assimilation (A) or food digested was estimated by subtracting F from C. Larval growth (P) was estimated by subtracting the initial dry weight of the larvae in each instar from the final dry weight of the respective instars. Metabolism (R) was estimated as difference between larval growth (P) and assimilation (A). All these parameters under investigation were expressed in dry weight. Rates of consumption (Cr), assimilation (Ar), growth (Pr) and metabolism (Mr) were calculated by dividing the respective amounts of weight by the product of mid body weight (g) of the larva and duration (days) required for the completion of respective instar (Waldbauer, 1968). The mean larval weight of the respective instars was treated as mid body weight.

The rates are expressed in terms of g/g live larvae/day.

$$\text{Consumption rate (Cr)} = C/\{\text{Mid body weight (g)} \times \text{Duration (days)}\}$$

$$\text{Assimilation rate (Ar)} = A/\{\text{Mid body weight (g)} \times \text{Duration (days)}\}$$

$$\text{Growth rate (Pr)} = P/\{\text{Mid body weight (g)} \times \text{Duration (days)}\}$$

$$\text{Metabolic rate (Mr)} = R/\{\text{Mid body weight (g)} \times \text{Duration (days)}\}.$$

TABLE 1: Food consumption, utilization, growth and relative rates in young instars of silkworm selected for high, medium and low lines of NB18

Larval instars	Lines	C (g)	A (g)	P (g)	R (g)	Cr (g/g/day)	Ar (g/g/day)	Pr (g/g/day)	Mr (g/g/day)
I	High	0.003 ±0.0004	0.0011 ±0.0002	0.0006 ±03	0.0005 ±0.0001	1.5832 ±0.1658	0.5916 ±0.0755	0.3128 ±0.0066	0.2789 ±0.0762
	Medium	0.0027 ±0.0005	0.001 ±0.0002	0.0006 ±03	0.0004 ±0.0002	1.3811 ±0.2652	0.5115 ±0.1058	0.3151 ±0.0035	0.1964 ±0.1063
	Low	0.0021 ±0.0005	0.0008 ±0.0002	0.0005 ±03	0.0003 ±0.0002	1.289 ±0.3069	0.491 ±0.1228	0.3192 ±0.0123	0.1719 ±0.1171
	Control	0.0022 ±0.0005	0.0008 ±0.0002	0.0003 ±03	0.0005 ±0.0001	1.7823 ±0.2457	0.6659 ±0.0813	0.2784 ±0.0129	0.3875 ±0.0917
CD ($P \leq 0.05$)		0.0009	0.0003	0.0001	0.0003	0.4230	0.1845	0.0188	0.1864
II	High	0.0207 ±0.0015	0.0077 ±0.0006	0.004 ±0.0002	0.0037 ±0.0006	0.9023 ±0.0791	0.3345* ±0.0256	0.1729* ±0.0021	0.1616 ±0.0263
	Medium	0.015* ±0.001	0.0056 ±0.0006	0.003 ±0.0001	0.0026 ±0.0007	0.8003 ±0.0632	0.2989 ±0.0357	0.16 ±0.0057	0.1389* ±0.0366
	Low	0.0072 ±0.0006	0.0027 ±0.0002	0.0025 ±03	0.0002 ±0.0002	0.468 ±0.039	0.1755 ±0.013	0.1625 ±03	0.013 ±0.013
	Control	0.0075 ±0.0008	0.0028 ±0.0003	0.0024 ±0.0001	0.0004 ±0.0003	0.4975 ±0.0777	0.1857 ±0.0291	0.1607 ±0.0082	0.0251 ±0.0219
CD ($P \leq 0.05$)		0.0019	0.0008	0.0003	0.0008	0.1263	0.0498	0.0084	0.0498

C-Consumption, A-Assimilation, P-Larval growth, R-Metabolism

Cr-Consumption rate, Ar-Assimilation rate, Pr-Larval growth rate, Mr-Metabolic rate

TABLE 2. Food consumption, utilization, growth and relative rates in late instars of silkworm selected for high, medium, and low lines of NB18

					0.0803	0.3508	0	0.0716	
					0.011	0.0224	0	0.0001	
					0.0893*	0.4068*	0	0.0635	0893
					0.0053	0.0268	0	0.0045	0104
					0.0363	564	0		72
					0.0087	0302	0	03	13
					0.0617	0.0324	0.1322	0.06	
					0.0097	0.0218	0.008	0.00	

TABLE 3. Food consumption, utilization, growth and relative rates in young instars of silkworm selected for high, medium and low lines of pure mysore

Larval instars	lines	C (g)	A (g)	P (g)	R (g)	Cr (g/g/day)	Ar (g/g/day)	Pr (g/g/day)	Mr (g/g/day)
I	High	0.0026* ±0.0002	0.001* ±0.0001	0.0004 ±0.0000	0.0005 ±0.0001	1.713 ±0.1019	0.6452 ±0.0385	0.2803 ±0.0000	0.3648 ±0.0385
	Medium	0.0021 ±0.0002	0.0008 ±0.0001	0.0003 ±0.0000	0.0004 ±0.0001	1.8144 ±0.2426	0.6725 ±0.0831	0.282 ±0.0071	0.3905 ±0.0893
	Low	0.0018* ±0.0002	0.0007* ±0.0001	0.0003 ±0.000	0.0004 ±0.0001	1.8037 ±0.1154	0.6815 ±0.0616	0.2613* ±0.0057	0.4202 ±0.0646
	Control	0.0021 ±0.0002	0.0008 ±0.0001	0.0003 ±0.0000	0.0004 ±0.0001	1.7391 ±0.1285	0.6452 ±0.0486	0.2861 ±0 3	0.359 ±0.0486
CD ($P \leq 0.05$)		0.0003	0.0001	0.0000	0.0001	0.2965	0.1130	0.0090	0.1191
II	High	0.0067* ±0.0002	0.0025* ±0.0001	0.0014* ±0.0001	0.0024* ±0.0001	0.6024* ±0.0051	0.2266* ±0.0006	0.1252 ±0.0045	0.2177* ±0.0003
	Medium	0.0059* ±0.0002	0.0022* ±0.0001	0.0012* ±0.0001	0.0011* ±0.0001	0.6115* ±0.0212	0.2301* ±0.006	0.1201 ±0.0022	0.11* ±0.0081
	Low	0.0042* ±0.0002	0.0016 ±0.0001	0.0009* ±0.0001	0.0006 ±0.0001	0.527 ±0.0295	0.1982 ±0.0126	0.1157 ±0.0032	0.0761 ±0.0153
	Control	0.0045 ±0.0002	0.0017 ±0.0001	0.0011 ±0.0001	0.0006 ±0.0001	0.4807 ±0.0186	0.1793 ±0.0050	0.1181 ±0.0045	0.0612 ±0.009
CD ($P \leq 0.05$)		0.0003	0.0001	0.0001	0.0001	0.0377	0.0188	0.0067	0.0188

C-Consumption, A-Assimilation, P-Larval growth, R-Metabolism, Cr- Consumption rate, Ar-Assimilation rate, Pr-Larval growth rate, Mr-Metabolic rate.

TABLE 4. Food consumption, utilization, growth and relative rates in late instars of silkworm selected for high, medium and low lines of pure mysore

Larval instars	Lines	C (g)	A (g)	P (g)	R (g)	Cr (g/g/day)	Ar (g/g/day)	Pr (g/g/day)	Mr (g/g/day)
III	High	0.029 ±0.003	0.0[illegible] ±0.0[illegible]	0.0[illegible] ±0.0[illegible]	[illegible]	0.345 ±0.04	[illegible]	0.09 ±0.00	[illegible]
	Medium	0.025 ±0.00	0.0[illegible] ±0.0[illegible]	0.0[illegible] ±0.0[illegible]	[illegible]	0.3[illegible] ±0.02[illegible]	0.12[illegible] ±0.01	0.0[illegible] ±0.00[illegible]	[illegible]
	Low	0.016 ±0 [illegible]	[illegible]	0.0056 ±0.0002	0.0004 ±0.0002	0.2[illegible]12 ±0 [illegible]3	0.094 ±0 [illegible]	0.0[illegible] ±0.0[illegible]	0.00[illegible] ±0.0[illegible]
	Control	0.019 ±0.001	[illegible]	[illegible]	[illegible]	0.2[illegible]41 ±0.03	0.10 ±0.01	0.0[illegible] ±0.00	0.01 ±0.01
CD (*P* < 0.05)		0.004	0.00[illegible]8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
IV	High	0.14 ±0.0[illegible]	0.05 ±0.00	0.03 ±0.002	[illegible]	0.2[illegible]12 ±0.0[illegible]	0.105 ±0.00	0.06 ±0.0[illegible]	[illegible]
	Medium	0.128 ±0.0032	0.0483 ±0.0012	[illegible]	[illegible]	0.2[illegible]04 ±0.0[illegible]	0.094 ±0.00	0.0[illegible]26 ±0.0[illegible]	0.02 ±0.00
	Low	0.1[illegible]17 ±0.0068	0.042 ±0.0026	0.0[illegible] ±0.0[illegible]	0.0097 ±0.00	0.250 ±0.0422	[illegible]	[illegible]	0.0[illegible] ±0.0[illegible]
	Control	0.127 ±0.00[illegible]9	0.04[illegible]7 ±0.00[illegible]1	0.0357 ±0.00[illegible]5	0.012 ±0.00[illegible]6	0.22 ±0.0[illegible]64	[illegible]	[illegible]	0.0[illegible] ±0.01
CD (*P* < 0.05)		[illegible]	[illegible]	[illegible]	0.0110	[illegible]	[illegible]	0.0040	[illegible]
V	High	2.0 ±0.09	[illegible]	[illegible]	[illegible]	0.4[illegible] ±0.02	[illegible]	[illegible]	0.[illegible]6 ±0.[illegible]0
	Medium	[illegible]04 ±0.033	0.639 ±0.012	0.262 ±0.00[illegible]	0.377 ±0.0[illegible]54	0.3647 ±0.0114	0.1368 ±0.0043	0.0[illegible] ±0.00[illegible]	0.[illegible] ±0.[illegible]
	Low	[illegible]	[illegible]	0.199 ±0.052	0.[illegible]697 ±0.[illegible]605	0.[illegible]12 ±0.[illegible]29	0.1617 ±0.03[illegible]6	0.054 ±0.00	0.0[illegible] ±0.04[illegible]
	Control	[illegible]	[illegible]	0.242 ±0.023	0.4[illegible] ±0.0[illegible]	0.4051 ±0.00[illegible]	0.15[illegible]9 ±0.00[illegible]7	0.054[illegible] ±0.00	0.09[illegible] ±0.0034
CD (*P* < 0.05)		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

C-Con[illegible] A [illegible] R [illegible]
Mr-M[illegible]

RESULTS AND DISCUSSION

Food consumption and utilization were low in young instars and increased gradually as the growth progressed in both selected and control lines of NB18 and Pure Mysore (Tables 1–4). The food consumption and utilization were higher in NB18 than Pure Mysore. These results in general are in agreement with the observation of earlier workers (Yamamoto and Fujimaki, 1982; Benchamin and Jolly, 1984; Periasami and Radhakrishnan, 1985; Remaderi *et al.*, 1991; Remadevi *et al.*, 1992). However the food consumption among the selection lines varied significantly in both the strains. In NB18, food consumption, assimilation and metabolism were significantly ($P \leq 0.05$) higher in high and medium lines of II, III, IV, and V instars and significantly ($P < 0.05$) lower in low lines of III and IV instars than control. The growth was significantly ($P < 0.05$) higher in high and medium lines of I, II, III IV instars and high line of IV instar, however it was significantly ($P < 0.05$) lower in low lines of III and IV instars than control (Tables 1 & 2). Whereas in Pure Mysore, significantly ($P \leq 0.05$) lower consumption was noticed in low lines of I, II, IV and V instars than control. However when compared to control, the consumption and assimilation were found to be higher in high lines of I, IV and V instars and high and medium lines of II and III instars. The growth was higher in high lines of II, III and medium lines of III and lower in low lines of II and V instars (Tables 3 & 4). A similar results have also been reported in mice (Fowler, 1962; Lang and Legates, 1969) and in chicken (Pym and Nicholas, 1979; Siegel *et al.*, 1984). These results indicate that genetic alterations implemented by selection for pupal weight modified food utilization and growth in silkworms.

The relative rates of consumption (Cr) and assimilation (Ar) in the selected and control lines of NB18 Pure Mysore were higher during early instars than late instars and decreased till fourth instar and again increased in fifth instar (except fourth instar of low line in NB18) (Tables 1–4). Present findings corroborates with the observation of Ueda and Suzuki (1967), Ueda *et al.* (1971), Mathavan *et al.* (1987). Decrease in relative rates in late instars are partly due to disproportionate increase in less metabolically active tissues such as lipid reserves (Slansky and Scriber, 1985) and increase of rates in fifth instar may be due to physiological functions of an insect. The percentage of increase in Cr observed in fifth instar of NB18 was 46, 6, 81 and 69 while in pure Mysore it was 43, 46, 72, 80 in high, medium, low and control lines respectively (Tables 2 & 4). The percentage of increase in metabolic rate in fifth instar of NB18 was 60, 18, 67 and 35 whereas in Pure Mysore it was 59, 73, 79 and 75 in high, medium, low and control lines respectively (Tables 2 & 4). Selection for pupal weight in silkworms favoured the low selection lines for Cr, Ar and Mr in fifth instar both in NB18 and Pure Mysore (Tables 2 & 4).

Growth rate (Pr) decreased with the progress of larval development in both NB18 and Pure Mysore. The percentage of reduction of Pr in fifth instar for NB18 was 29, 16, 30 and 33 whereas, in Pure Mysore it was 17, 23, 24 and 22 in high, medium, low and control lines respectively (Tables 2 & 4). The magnitude of Pr was higher in high and medium lines of NB18 than low and control lines (Tables 1 & 2). These results indicate that the rate of growth may partly be associated with the body size.

A similar observation have also been made by Medrano and Gall (1976) in the lines selected for 21 day pupal weight in *Tribolium castaneum*. However, similar situation does not exist in Pure Mysore, where the difference between growth rate and body size was negligible. It could be attributed that lines selected for pupal weight did not show significant difference by virtue of selection, probably due to low genetic variability for pupal weight in the base population. This was apparently resulted in low degree of selection response in case of pure Mysore (Puttaraju and Rajanna, 1997). These results suggest that eight generations of selection for pupal weight altered food utilization, growth and relative rates in silkworms and it is necessary to take into account of such factors in future breeding programmes. Selection with the aid of physiological analysis may help to throw light on the underlying genetic situations controlling growth and food utilization.

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REFERENCES

Benchamin, K. V. and Jolly, M. S. (1984) Studies on ingestion, digestion and conversion efficiency in polyvoltine and bivoltine silkworm breeds (*B. mori* L.), *Proc. III Oriental Entomology Symposium, Trivandrum* **2**: 195–206.

Fowler, R. E. (1962) The efficiency of food utlization, digestibility of food stuffs and energy expenditure of mice selected for large or small body size, *Gen. Res. Comb.* **3**: 51–68.

Horie, Y. and Watanabe, K. (1983) Daily utilization and consumption of dry matter in food by the silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae), *Appl. Entomol. Zool.* **18**: 70–80.

Krishnaswami, S. (1978) New Technology of Silkworm Rearing. CSR & TI. Bulletin No. 2, Central Silk Board, India, **37(1)** p. 51.

Lang, B. J. and Legates, J. E. (1969) Rate, composition and efficiency of growth in mice selected for large and small body weight, *Thero. Appl. Genet.* **39**: 306–314.

Magadum, S. B., Remadevi, O. K., Shivashankar, N. and Datta, R. K. (1966a) Nutritional indices on some bivoltine breeds of silkworm, *Bombyx mori* L., *Indian. J. Seric.* **35(2)**: 95–98.

Magadum, S. B., Remadevi, O. K., Datta, R. K. and Shivashankar, N. (1966b) Correlation between various nutritional parameters of silkworm, *Bombyx mori* L. during V instar, *Indian. J. Seric.* **35(2)**: 138–141.

Mathavan, S. and Pandian, T. J. (1974) Use of faecal weight as an indicator of food consumption in some lepidopterans, *Oecologia (Berl.)* **15**: 177–185.

Mathavan, S., Santhi, G. and Nagaraja Sethuraman, B. (1987) Effects of feeding regime on energy allocation to reproduction in the silkworm *Bombyx mori* L., *Proc. Indian. Acd. Sci. (Animal Sci.)* **96(3)**: 333–340.

Matsumura, S., Takeuchi, Y. and Kousaka, T. (1955) Studies on nutrition of the recent commercial races of silkworm *Bombyx mori* L., *Tech. Bull. Seri. Exp. Stn.* **60**: 1–47.

Madrano, J. F. and Gall, A. E. (1976) Food consumption, feed efficiency, metabolic rate and utilization of glucose in lines of *Tribolium castaneum* selected for 21 day pupa weight, *Genetics* **83**: 393–407.

Periasami, K. and Radhakrishnan, R. (1985) Quantitative study of food utilization and silk production in *Bombyx mori* L. for evaluation of superior varieties, *Sericologia* **25(4)**: 491–500.

Petrusewicz, M. and Macfadyen, K. (1970) *Productivity of Terrestrial Animals*, IBP Hand Book No. 13. Blackwell Scientific Publications: Oxford, p 190.

Puttaraju, H. P. and Rajanna, K. L. (1997) Short term selection for pupal weight in the silkworm *Bombyx mori* L.-1. Direct response, *Indian. J. Seric* **36(2)**: 121–127.

Pym, R. A. E and Nicholas, A. J. (1979) Selection for food conversion in broilers. Direct and correlated responses to selection for body weight gain, food consumption and food conversion ratio, *Br. Poult. Sci.* **20**: 73–86.

Remadevi, O. K., Magadum, S. B., Shivashankar, N., Benchamin, K. V. and Datta, R. K. (1991) Studies on the growth rate and growth pattern in the fifth instar larvae of some multivoltine races of silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae), *Indian J. Seric.* **30(2)**: 125–130.

Remadevi, O. K., Magadum, S. B., Shivashankar, N. and Benchamin, K. V. (1992) Evaluation of food utilization efficiency in some polyvoltine breeds of silkworm *Bombyx mori* L., *Sericologia* **32(1)**: 61–66.

Roberts, R. C. (1973) Food consumption in mice selected for high and low body weight, *Genetics* **74(1&2)**: Suppl. 232.

Siegel, P. B., Cherry, J. A. and Dunnington, E. A. (1984) Feeding behaviour and feed consumption in chickens selected for body weight, *Ann. Agric. Fenn.* **23**: 247–252.

Slansky, F. Jr. and Scriber, J. M. (1985) Food consumption and utilization. In: *Comprehensive Insect Physiology*, Vol. 4. Kerkut, G. A. and Gilbert, L. I.: (Editors). Pergamon Press: Oxford, 87–163.

Ueda, S. (1965) Changes in some quantitative factors and their mutual relationships concerning the growth and development in fifth larval instar of the silkworm, *Bombyx mori* L., *Bull. Seri. Exp. Stn.* **19(4)**: 342–343.

Ueda, S. and Suzuki, K. (1967) Studies on the growth of the silkworm, *Bombyx mori* L. I. Chronological changes of the amount of food ingested and digested, body weight and water content of body and their mutual relationships, *Bull. Sericul. Exp. Stn.* **22(1)**: 65–67.

Ueda, S., Kimura, R. and Suzuki, K. (1971) Studies on the growth of the silkworm, *Bombyx mori* L. III. Relative increase in body weight and silk gland weight in the fifth instar larvae, *Bull. Seric. Exp. Stn. Jpn.* **25**: 1–20.

Ueda, S. (1982) Theory of the growth of silkworm larvae and its applications, *JARQ* **15(3)**: 180–184.

Waldbauer, G. P. (1968) The consumption and utilization of food by insects, *Advances in Insect Physiology* **5**: 229–288.

Yamamoto, T. and Fujimaki, T. (1982) Interstrain difference in food efficiencies of the silkworm *Bombyx mori* L. reared on artificial diet, *J. Seric. Sci. Jpn.* **51(4)**: 312–315.

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New Species of Gall Mites (Acari: Eriophyidae) from South India-I

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ABSTRACT. Five species of gal forming eriophyid mites treated in this paper are all new to science. Suitable line drawings and host records are provided.
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KEYWORDS: Gall mites, galls, erineum, pouch galls, Aceria.

INTRODUCTION

Periodical collections and study of phytophagous mites from various places of South India have revealed the presence of several new species of gall mites. The types and paratypes have been deposited in the Department of Agricultural Entomology collections, Tamil Nadu Agricultural University (TNAU). The abbreviations used in the figures are: AP 1-Internal female Apodeme; CS-Side view of Cauda; DA-Dorsal view of Anterior end; ES-Side skin structure; F-Feather claw; GF 1-Female genitalia and coxal bases; GM-Male genitalia; L1-Left foreleg; L2-Left hindleg; S-Side view of mite.

SYSTEMATICS

Subfamily: Eriophyidae

1. Aceria allophylae sp. nov. (Fig. 1)

Diagnosis: The general features of this mite resembles *Aceria sapindi* Channabasavanna (1966) but differs by its shield design, length of second ventral seta, absence of accessory seta, clear coxal bases and five rayed feather claw.

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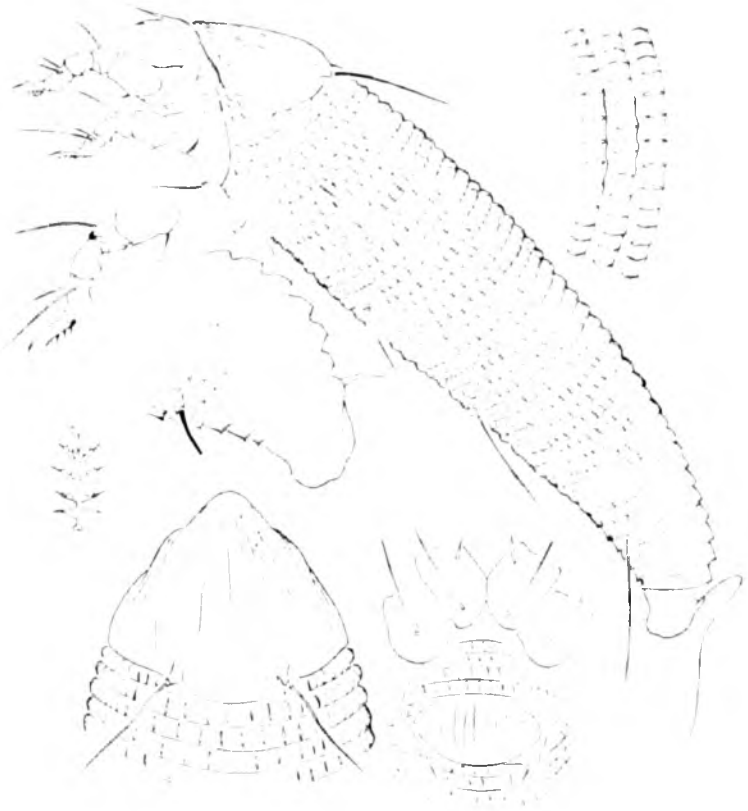

FIGURE 1

Female: 185–190 long, 44 wide, 35 thick, whitish, wormlike. Rostrum 16 long, pointing obliquely forward. Shield 25 long, 32 wide, shield area comprising media, admedian and several submedians; anterior one-third of shield area filled with dots or dashed. Dorsal tubercles 15 apart; dorsal setae 32 long, arching backward. Foreleg 30 long; tibia 5 long; tibial seta missing; tarsus 6 long; claw 8 long tapering. Feather claw 5 long, 5 rayed. Hindleg 26 long; tibia 4 long, tarsus 5 long; claw 6 long; coxal setation regular; seta on tubercle I, 12 long; seta on tubercle II, 25 long; seta on tubercle III, 38 long; bases of coxae smooth.

Abdominal thanosome with about 63 rings; dorsum of telosome free of microtubercles; telosome with microstriae. Lateral seta 15 long on ring 6; first ventral seta 30 long on ring 22; second ventral seta 40 long on ring 38; third ventral 30 long on ring

5 from behind; accessory seta missing. Female genitalia 10 long, 20 wide; coverflap with 10 longitudinal ribs. Genital seta 5 long.

Male: Not known.

Types: A holotype slide with females. Tamil Nadu: Kolli Hills, Salem. 30.v.1995, ex *Allophylus* sp. (Sapindaceae). Coll. G. Umapathy, Coll. No. 170 (TNAU).

Relation to host: The whitish wormlike mites are leaf vagrants.

2. *Aceria apodytae sp. nov. (Fig. 2)*

Diagnosis: The general features of this mite resembles *Aceria gastrotrichus* (Nalepa, 1918) in its shield design and general features. The new species differs from it by the shield designtern involving numerous straight lines; close-set; the admedian and several submedians could be located among faint lines. The coverflap scorings also very slightly apart from the measurements.

Female: 170–180 long, 52 wide, 50 thick, whitish, wormlike. Rostrum 20 long, pointing obliquely forward. Antapical seta absent. Shield 26 long, 38 wide, shield area completely packed with an array of fading close-set fine lines; few submedians are strongly produced. Dorsal tubercles 15 apart, arising from rear shield margin; dorsal setae 25 long, diverging backward. Foreleg 30 long; tibia 5 long; tibial seta 4 long placed at upper one third; tarsus 7 long; claw 7 long; sharp. Feather claw 5 long and 5 rayed. Hindleg 26 long; tibia 4 long and tarsus 6 long; claw 6 long; legs setation regular; first setiferous tubercles slightly above and away from forecoxal junction; second setiferous tubercles placed ahead of line across third setiferous tubercle; bases of coxae clear.

Abdominal thanosome with about 85 uniform rings. Micro-tubercles large and oval, present between ring margins. Lateral seta 18 long on ring 12, first ventral seta 34 long on ring 32; second ventral seta 42 long on ring 52; third ventral 25 long on ring 5 from behind; accessory seta missing. Female genitalia 13 long, 16 wide away from coxal bases; coverflap with 8 major ribs, few dashes in between. Genital seta 10 long.

Male: Not known.

Types: A holotype and there paratype slides Tamil Nadu: Courtallam forest range, Tirunelveli, 20.i.1995, ex *apodytes* sp. (Icacinaceae) Coll. G. Umapathy, Coll. No. 138 (TNAU).

Relation to host: The whitish wormlike mites thrive within the pouch galls.

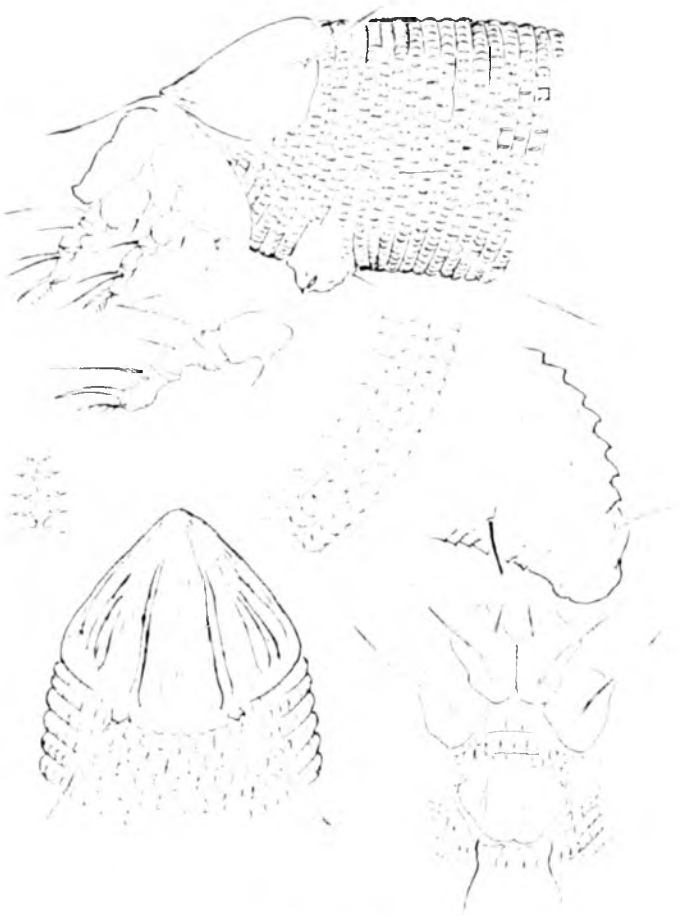

FIGURE 2

3. Aceria ariyankavensis sp. nov. (Fig. 3)

Diagnosis: The new species resembles *Aceria ruelliae* Channabasavanna (1966) but differs in its shield design, absence of accessory seta, very long opisthosomal setae, design at coxal bases and six rayed featherclaw.

Female: 160 long, 42 wide, 32 thick, pale brown, wormlike-mites. Rostrum 22 long, pointing obliquely forward. Antapical seta 5 long. Shield 30 long, 38 wide, shield area with a median, distinctly seen beyond anterior half of shield; an admedian and two submedians present. Anterolateral shield area with traces of fine granulations. Dorsal tubercles 18 apart; dorsal setae 20 long, diverging backward. Foreleg 30 long; tibia 7 long; tibial seta 4 long placed at centre; tarsus 7 long; claw 8 long, arched, tapering

FIGURE 3

and sharp. Feather claw 6 rayed. Hind leg 25 long; tibia 5 long and tarsus 4 long; Fore coxae join anteriorly; leg setation regular; bases of coxae with dashes; setation normal; seta on tubercles I, 15 long; seta on tubercles II, 30 long; seta tubercles III, 40 long.

Abdominal thanosome with about 62 rings, microtubercles circular in shape, set on rear ring margins; telosomal rings with microstriae. Lateral seta 40 long on ring 10; first ventral seta 30 long on ring 28; second ventral seta 30 long on ring 42; third ventral 40 long on ring 5 from behind; caudal seta about 50 long; accessory seta missing. Female genitalia 14 long, 15 wide, coverflap with 16 longitudinal ribs. Genital seta 30 long.

Male: Not known.

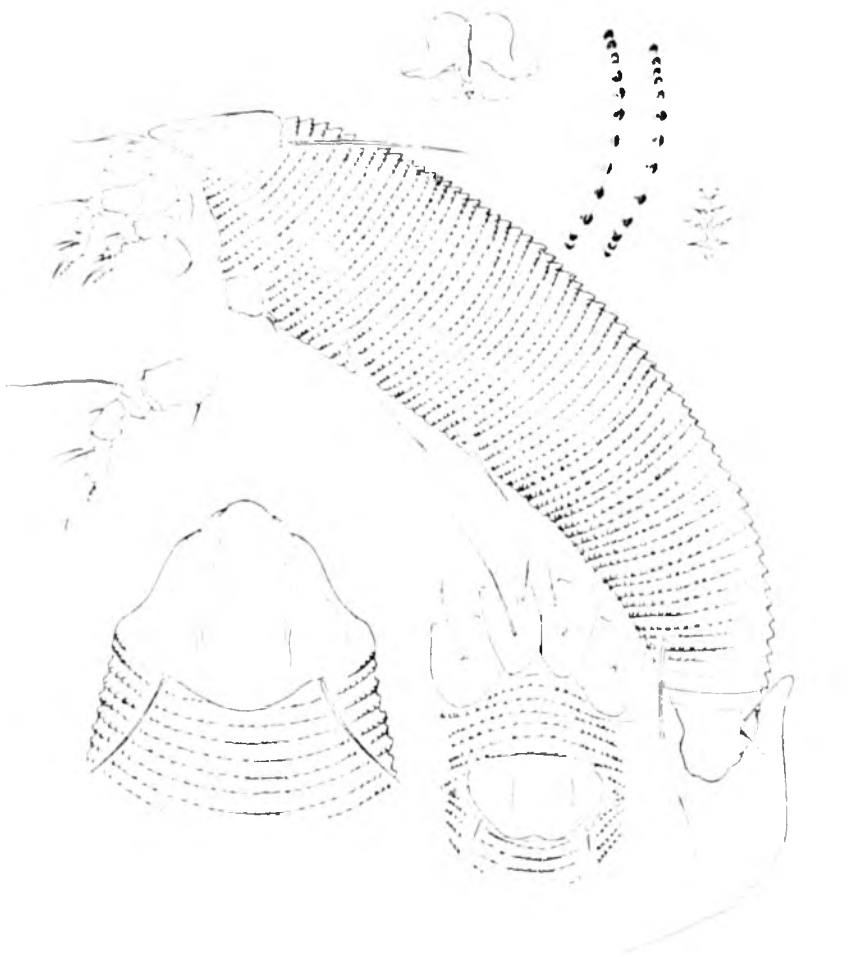

FIGURE 4

Types: A holotype slide with females and three paratype slides. Kerala: Ariyankavu forest, 10.vi.1995, ex *unidentified tree* (F: unknown). Coll. G. Umapathy, Coll. No. 189 (TNAU).

Relation to host: The mite infestation causes severe deformation of leaves. Fine erineal hairs present in the wavy pouches.

4. Aceria articulate sp. nov. (Fig. 4)

Diagnosis: The shield pattern, in general, resembles *Aceria commelinae* (Mohanasundaram, 1980) and differs from it by the absence of median line, clear coxal bases and four rayed feather claw.

FIGURE 5

Female: 215 long, 45 wide, 42 thick, wormlike whitish mites. Rostrum 17 long, downcurved; antapical 3 long. Shield 25 long, 35 wide, shield area with admedian and several submedians. Dorsal tubercles 15 apart, arising from posterior shield margin; dorsal setae 35 long, arching backward. Foreleg 35 long; tibia 4 long; tibial seta 5 long; tarsus 6 long; claw 8 long tapering, sharp. Feather claw 5 long and 4 rayed. Hindleg 30 long; tibia 3.5 long and tarsus 4 long; legs setation normal. Fore coxae joins anteriorly; bases of fore coxae slightly connate; first setiferous tubercles well above fore coxal approximation; seta I, 15 long; second setiferous tubercles well ahead of line across third setiferous tubercles; seta II, 35 long, seta III 40 long; bases of coxae without scoring.

Abdominal thanosome with about 54 rings; micro-tubercles rear set, suppressed or faded at telosome. Lateral seta 12 long on ring 8; first ventral seta 45 long on ring

20; second ventral seta 5 long on ring 32; third ventral 50 long on ring 6 from behind; Accessory seta 7 long. Female genitalia 10 long, 20 wide; coverflap with 8 ribs; genital seta 10 long.

Male: 205 long, 55 wide. Genitalia 15 wide, seta 10 long.

Types: A holotype slide with males and females and five paratype slides with females. Tamil Nadu: Kolli hills, Salem, 29.v.1995, ex *Borreria articulans* F. Williams (Rubiacaeae) Coll. G. Umapathy, Coll. No. 167 (TNAU).

Relation to host: The mite infestation causes erineum patches on the lower leaf surface.

5. *Aceria attakattiensis sp. nov.* (*Fig. 5*)

Diagnosis: The new species resembles *Aceria astibonis* Keifer (1960) *A. daleae* and *A. byersi* (Keifer, 1961) in its general features and differs from *A. daleae* due to unbranched admedian and submedians, circular microtubercles, presence of accessory seta, clear genital coverflap and six rayed feather claw.

Female: 125–130 long, 30 wide, wormlike whitish mites. Rostrum 12 long, downcurved; antapical 4 long. Shield 20 long, 23 wide, shield area with a median forked at the middle, two submedians present. Dorsal tubercles 12 apart, shield seta 18 long arising from rear shield margin, arching back. Foreleg 28 long; tibia 4 long; tibial seta 5 long, centrally placed; tarsus 5 long; claw 6 long tapering, sharp. Feather claw simple, 6 rayed, rays spread broadly. Hindleg 26 long; tibia 4 long, tarsus 4.5 long; Coxae broadly joined with a fore sternal line Coxae with usual setiferous tubercles. First setiferous tubercles far away and well above fore coxal junction; second setiferous tubercles ahead of transverse line across third setiferous tubercles; bases of coxae with faint dots and short stripes.

Abdominal thanosome with about 68 microtuberculate rings. Micrtotubercles circular or oval, large, set or rear ring margins, telosomal rings beyond third ventral seta with microstriae. Lateral seta 12 long on ring 8; first ventral seta 20 long on ring 15; second ventral seta 16 long on ring 28; third ventral seta 22 long on ring 5 from behind; Caudal seta 35 long. Accessory seta 3 long. Female genitalia 12 long, 17 wide; coverflap smooth; genital seta 8 long.

Male: 120 long, 30 thick. Genitalia 15 wide, seta 6 long.

Types: A holotype slide with males and females and two paratype slides with females. Tamil Nadu: Attakatti forest, Coimbatore, 15.i.1994, ex *Asystasia* sp. (Acanthaeae). Coll. G. Umapathy, Coll. No. 20 (TNAU).

Relation to host: The mite infestation results in erineum formation.

REFERENCES

Channabasavanna, G. P. (1966) *A Contribution to the Knowledge of Indian Eriophyid Mites (Eriophyoidea: Trombidiformes: Acarina)*, University of Agricultural Sciences: Hebbal, Bangalore, India, 154 pp.

Nalepa, A. (1918) Eriophyiden aus Java. I., *Verh. Zool. Bot. Ges. Wien.* **68**: 43–45.

Keifer, H. H. (1960) Eriophyid studies B-1, *Bur. Entomol., Cal. Dept. Agr.* 20.

Keifer, H. H. (1961) Eriophyid studies, *Bur. Entomol., Cal. Dept. Agr.* 20.

Mohanasundaram, M. (1980) Indian eriophyid studies II, *Mysore J. Agric. Sci.* **14**: 515–528.

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Karyological Study in *Ergolis merione* Cram. (Nymphalidae: Lepidoptera) With Indications of Localized Centromeres and Female Heterogamety

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ABSTRACT: A study of mitotic and meiotic chromosomes was carried out in *Ergolis merione* Cram. (Nymphalidae: Lepidoptera) using brain ganglia of caterpillars of both the sexes as well as the testicular tissue of males. The diploid chromosome number was found to be 54. By the application of colchicine treatment, primary constrictions were recognized on the chromosomes. It was thus possible to arrange the chromosomes on the basis of the centromeric positions. The fifty four chromosomes of the diploid set were arranged into six metacentric, three submetacentric, one subtelocentric and sixteen acrocentric autosomal pairs. Female heterogamety was clearly evident form the heteromorphic ZW sex chromosome pair comprising a largest submetacentric Z and a smallest metacentric W chromosome. Diakinetic figures in the colchicinized insects showed discrete chiasmata in the bivalents. The issue of the nature of the centromeres in Lepidoptera has been discussed in the light of the present results. © 2000 Association for Advancement of Entomology

KEYWORDS: Lepidoptera, centromeres, heterogamety, sex chromosomes.

INTRODUCTION

There has been a great difficulty in discerning the Lepidopteran chromosomes individually from the metaphase plates because of their extremely small size and almost dot like isodiametric morphology (Robinson, 1971) with round or somewhat oval shape in polar view (Saitoh *et al.*, 1992; Saitoh and Abe, 1994). Thus their identification and arrangement into homologues could not be possible. Moreover, a controversy exists even with regard to their centromeric organization. Lepidopteran chromosomes have generally been described as holocentric or having diffused centromeres (Barry *et al.*, 1967; Bauer, 1967; Maeki, 1980; Murakami and Imai, 1974; Suomalainen, 1953, and others), yet in the opinion of White (1973) the status of centromeres is still somewhat doubtful. Some of the recent work (Bigger, 1975, 1976; Rishi and Rishi, 1979, 1981,

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1990), has shown primary constrictions and evidence has also been provided by electron microscopy (Gassner and Klemetson, 1974). Another problem in Lepidoptera is the identification of the sex chromosomes. The sex chromosomes of most lepidopteran species are hardly distinguishable from the autosomes in metaphase plates, because their sizes and shapes are usually the same. Further the Z and W chromosomes of most species are morphologically identical in size, forming a bivalent that is not recognizable by heteromorphism either. Some lepidopteran species are known which posses clear female heterogamety (Bigger, 1975, 1976; Kawamura and Niino, 1991; Maeki, 1981; Rishi and Rishi, 1990; Suomalainen, 1969, 1971; Traut, 1986; Traut *et al.*, 1986).

During the present investigation, in addition to the male gonadal tissue, somatic tissue of brain was also analyzed in both the sexes by employing colchicine—air drying technique so that much improved detail regarding the centromeric nature has been obtained and female heterogamety has been clearly evident.

MATERIAL AND METHODS

Different instar larvae of *Ergolis merione* were collected from wild plants of *Ricinus communis* (Arind) in the vicinity of Kurukshetra University campus. They were fed in the laboratory and the prepupal stages were utilized for chromosomal preparation. The brain ganglia and prepupal testes were taken out in 0.7% sodium chloride solution in which 0.01 ml of 0.1% colchicine had been added and kept as such for 35–40 minutes. These tissues were then pretreated for 15 minutes in 1.0% hypotonic sodium citrate solution. Fixation was done in acetic alcohol (1 : 3) for 30 minutes and the tissue was teased into a suspension in a drop of 50% acetic acid on clean slides. The slides were left to dry in the air. These were then stained as such in 2% Giemsa solution.

RESULTS

The cells of brain ganglia of both male and female sexes as well as the male gonads yielded satisfactory results. Early metaphase plates from the brain tissue of female insects showed much elongated chromosomes (Fig. 1). These observations were extremely significant since the chromosomes showed distinct primary constrictions and clear cut splitting of the chromatids. It was thus possible to arrange the chromosomes in the form of a tentative karyotype on the basis of size measurements. Fifty two autosomal elements were arranged into twenty six homologues (Fig. 2). Thus there are six metacentric, three submetacentric, one subtelocentric and sixteen acrocentric pairs. The sex chromosomes were fairly distinct in size: Z was still longer than the largest autosomal while W was considerably the smallest element. Primary constrictions were recognized at the subcentral and central portions in the Z and W respectively. Female heterogamety was clearly evident from the heteromorphic sex chromosome pair comprising a largest submetacentric (Z chromosome) and a smallest metacentric (W chromosome). The ZZ sex pair in homogametic male comprised the largest submetacentrics (Fig. 4).

FIGURE 1. Somatic metaphase from brain cells of female *Ergolis merione* treated with 0.1% colchicine ($2n = 54$)

The spermatogonial metaphase from testes (Fig. 5), like the somatic metaphase chromosomes of brain ganglia, revealed separated chromatids. Thus the centromeric position could also be made out in these chromosomes. Very good diakinetic figures were available in the colchinized insects. There were twenty seven bivalents exhibiting discrete chiasmata (Fig. 6). The elements showed crosses, and end-to-end associations of the bivalents. As the diakinesis progressed, the chiasmata got terminalized and the bivalents were still more condensed so that at metaphase I (Fig. 7), they assumed the form of well defined dumb bell shaped elements. On an average, the chiasma frequency per bivalent comes to one in most of diakinetic stages.

DISCUSSION

Ergolis merione ($2n = 54$) described here cytologically for the first time, belongs to the largest family Nymphalidae of the sub division Rhopalocera of the order Lepidoptera. The most frequently occuring haploid number in this family is 30. The other common numbers are 29, 31, 33, 26 and 27. the wide range of variation

FIGURE 2. Karyotype prepared form Fig. 1.

in the chromosome numbers in this family cannot just be explained on the basis of the usual chromosomal changes. One tends to believe in the role of fusion and fragmentation (Bauer, 1967; Beliajeff, 1930; de Lesse, 1966; Federley, 1945; Gupta, 1964; Suomalainen, 1969; White, 1957).

Both types of kinetic organization—holokinetic as well as monokinetic have been claimed for Lepidoptera chromosomes. Holokinetic chromosome organization was deduced from missing primary constrictions and parallel separation in mitotic anaphase (Murakami and Imai, 1974), chromosome fragmentation and fusion in evolution giving rise to highly different chromosome numbers in related species (Suomalainen, 1953), the high doses of X-ray necessary to induce sterility (North and Holt, 1968), viability of chromosome fragments (Maeki, 1981) and the high rate of viability of X-ray induced translocation (Bauer, 1967).

A monokinetic organization of mitotic Lepidoptera chromosomes is derived from an electron microscopic study of *Ephestia and Trichoplusia* chromosomes (Gassner and Klemetson, 1974). A monokinetic structure of mitotic chromosomes is further deduced from the observation of primary constrictions in different species of *Pieris,*

FIGURE 3. Somatic metaphase from brain cells of male *E. merione* ($2n = 54$).

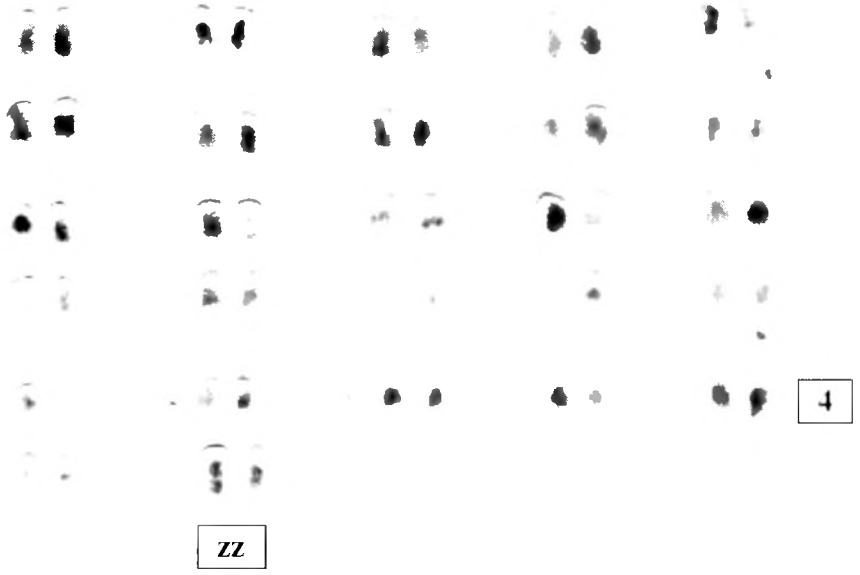

FIGURE 4. Karyotype prepared from Fig. 3.

FIGURE 5. A part of spermatogonial metaphase of *E. merione* showing splitting of chromatids.

FIGURE 6. Diakinesis of male *E. merione* ($n = 27$).

FIGURE 7. Metaphase I of male *E. merione* in polar view ($n = 27$).

Trabala vishnu and Scirpophaga auriflua (Bigger, 1975, 1976; Rishi and Rishi, 1979, 1981, 1990). Similar results with localized centromeres have been obtained by us during the present study in *E. merione* and the chromosomes are, therefore, grouped as metacentrics, sub-metacentrics and acrocentrics.

Fusions of monocentric chromosomes give rise to either very unstable dicentric chromosomes or by centric fusion (Robertsonian translocation) to stable monocentric (or at least monokmetic) chromosomes. Fusions of truly holokinetic (holocentric or polycentric) chromosomes can be regarded as centric fusions and are expected to behave as such. The very existence of chromosome fusions which are sufficiently stable to be maintained is an argument in favour of the interpretation that they are centric fusions (Rathjens, 1974). This would be expected only with holokinetic chromosomes. However, Rathjens (1974) and Traut (1986) cytologically determined high rates of breakage of the fusion chromosomes. These results are more in keeping with a behaviour of dicentric chromosomes and reflect a mitotic and/or meiotic instability of the fusion chromosome. They lead to the opposing conclusion that Lepidoptera chromosomes are functional monocentrics, at least those originally involved in the chromosome fusion. The present discovery of distinct localised centromeres in *Ergolis merione* is, thus, extremely interesting particularly with regard to the theories proposed in order to explain karyotypic evolution in Lepidoptera.

The presence of the constrictions on Lepidoptera chromosomes is therefore difficult to interpret. As metaphase progresses the chromosomes become more contracted and the constrictions cease to be visible. It was suggested (Bigger, 1975) that the primary centromere performed only a limited kinetic function early on in the mitotic cycle and this was superseded by the action of the holokinetic organisation of the chromosomes as they contract. Alternatively, the primary centromeres may not have any function at all other than holding the chromatids together at prometaphase but the disapperance of the constrictions early in metaphase may simply reflect the evolutionary change from monokinetic to holokinetic chromosomes. Even in the present investigations, the mitotic chromosomes of male brain ganglia are from the more advanced metaphase plate whee the chromosomes have become more contracted and the primary constrictions are not so clearly visible in some of the elements. However, the presence of the constrictions does make it possible to construct detailed karyotypes of the chromosome complements and helps in distinguishing between morphologically similar chromosomes.

The identification of sex chromosomes is only rarely possible in conventional mitotic metaphase chromosome spreads of Lepidoptera. It is characteristic of the chromosome sets of many lepidopteran species studied that, within a species, the chromosomes are approximately equal in size (Beliajeff, 1930; Federley, 1945; Lorkovic, 1941). Following the pioneer study with *Phragmatobia* by Seiler (1914), female heterogamety in Lepidoptera has been chromosomally demonstrated by recent workers providing revised and clear-cut evidence in the field (Bigger, 1975, 1976; Maeki, 1981; Rishi and Rishi, 1985, 1990; Suomalainen, 1965, 1969). Lepidopteran species are known which possess a bivalent which is clearly bigger than the others (de

Lesse, 1960; Suomalainen, 1965; Bauer, 1943) and White (1957) suggested that the big chromosome pair frequently found in the Lepidoptera is a sex chromosome pair, at least in those species which have a very high chromosome number. Suomalainen (1969) designated XY_1Y_2 for the females and XX for the males on the basis of sex chromosome trivalent in each of the four moth species studied by him. Most species possess a ZW : ZZ/XY : XX sex chromosome mechanism (Kawazoe, 1992; Maeki, 1981; Traut *et al.*, 1986). Female heterogamety with totally heterochromatic W element has been demonstrated by Maeki (1981) on the basis of C-band analysis. WZ bivalents of several moth and butterfly species have been recognised in the pachytene stage using light micorscopic techniques (Clarke *et al.*, 1977; Kawamura and Niino, 1991; Traut and Rathjens, 1973). Additionally W chromosome mutants have been visualized and measured by Marec and Traut (1994) and Traut *et al.* (1986) using electron microscopy of pachytene spreads.

The present study has resulted in the demonstration of the W- and Z-chromosomes based on karyotype analysis. Female is thus the heteromorphic sex possessing heteromorphic sex chromosome pair, Z being the largest and W the smallest of all the elements. *E. merione*, therefore, possess ZW : ZZ sex mechanism. These results are compatible with the report of Suomalainen (1969) showing that in four species of moths, the Z-chromosome was represented by the largest element in each.

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REFERENCES

Barry, B. D., Guthrie, W. D. and Dollinger, E. J. (1967) Evidence of a diffuse centromere in the European com borer, Ostrinia nubilalis (Lepidoptera: Pyralidae), *Ann. Entomol. Soc. Am.* **60(2)**: 487–488.

Bauer, H. (1943) Chromosomenforschung Fortschr, *Zool.* **7**: 256–287.

Bauer, H. (1967) Die kineticshe Organisation der Lepidopteren chromosomen, *Chromosoma (Berl.)* **22**: 101–125.

Beliajeff, N. K. (1930) Die chromosomenkomplexe und ihre Beziehung zur phylogenie bei den Lepidopteren, *Z. indukt. abstamm. u. Vereb. L.* **54**: 369–399.

Bigger, T. R. L. (1975) Karyotypes of some Lepidoptera chromosomes and changes in their holokinetic organization as revealed by new cytological techniques, *Cytologia* **40**: 713–726.

Bigger, T. R. L. (1976) Karyotypes of three species of Lepidoptera including an investigation of B chromosomes in *Pieris*, *Cytologia* **41**: 261–282.

Clarke, C., Mittwoch, U. and Traut, W. (1977) Linkage and cytogenetic studies in the swallowtail butterflies *Papilio polyxenes* Fab. and *Papilio machaon* L. and their hybrids, *Proc. R. Soc. B.* **198**: 385–399.

Federley, H. (1945) Die Konjugation der Chromosomen bei den Lepidopteren, *Soc. Sci. Fennica, Com. Biol.* **9**: 1–12.

Gassner, G. and Klemetson, D. J. (1974) A transmission electron microscope examination of hemipteran and lepidopteran gonial centromeres, *Can. J. Genet. Cytol.* **16**: 457–464.

Gupta, Y. (1964) Chromosome studies in some Indian Lepidoptera, *Chromosoma (Berl.)* **15**: 540–561.

Kawamura, N. and Niino, T. (1991) Identification of the Z-W bivalent in the silkworm, *Bombyx mori*. *Genetica* **83**: 121–123.

Kawazoe, A. (1992) Chromosomes of some univoltine moths in the cold season (3), *Chromosome Information Service* **53**: 27–30.

de Lesse, H. (1960) Speciation et variation chromosomique chez les lepidopteres Rhopaloceres, *Ann. Sci. Nat. Zool. Biol. Anim (Ser. 12)* **2**: 1–223.

de Lesse, H. (1966) Formules chromosomiques de Rhopaloceros D' Afrique, *Ann. Soc. Ent. Fr.* **2(2)**: 349–353.

Lorkovic, Z. (1941) Die chromosomenzahlen in der Spermatogenese der Tagfalter, *Chromosoma* **2**: 155–191.

Maeki, K. (1980) The kinetochore of the Lepidoptera. I. Chromosomal features and behaviour in mitotic and meiotic I cells, *Proc. Japan. Acad. Ser. B.* **56(8)**: 508–513.

Makei, K. (1981) Notes on the W chromosome of the Butterfly, *Graphium sarpedon* (Papilionidae: Lepidoptera), *Proc. Japan. Acad. Ser. B.* **57(10)**: 371–373.

Marec, F. and Traut, W. (1994) Sex chromosome pairing and sex chromatin bodies in W-Z translocation strains of *Ephestia kuehniella* (Lepidoptera), *Genome* **37(3)**: 426–435.

Murakami, A. and Imai, H.T. (1974) Cytological evidence for holocentric chromosomes of the silkworms, *Bombyx mori* and *B. mandarina* (Bombycidae: Lepidoptera), *Chromosoma* **46**: 167–178.

North, D. T. and Holt, G. (1968) Inherited sterility in progeny of irradiated male cabbage loopers, *J. Econ. Ent.* **61**: 928–931.

Rathjens, B. (1974) Zur Funktion des W-Chromatins bei *Ephestia kuehniella* (Lepidoptera). Isolierung and Charakterisierung von W-Chromatin-Mutaten, *Chromosoma* **47**: 21–44.

Rishi, S. and Rishi, K. K. (1979) Chromosomal analysis of *Trabala vishnu* Lef. (Lasiocampidae: Lepidoptera) with clear indications of localized centromeres, *Cytobios* **24**: 33–42.

Rishi, S. and Rishi, K. K. (1981) *On the Centromeric Organization in Lepidoptera. In Perspective in Cytology and Genetics 3*, Manna, G. K. and Sinha, U.: (Editors). Hindasia Publishers: Delhi, 69–72.

Rishi, S. and Rishi, K. K. (1985) Somatic and meiotic chromosomes analysis of *Danaus lemniace* Cramer (Lepidoptera: Nymphalidae), *Chromosome Information Service* **39**: 22–23.

Rishi, S. and Rishi, K. K. (1990) A chromosomal study of *Pieris brassicae* L. (Lepidoptera: Pieridae), *Cytobios* **64**: 203–207.

Robinson, R. (1971) *Lepidoptera Genetics*, 1st edition. Pergamon Press.

Saitoh, K. and Abe, A. (1994) Spermatocyte chromosomes of two fritillaries (Lepidoptera: Nymphalidae) of Japan, *Chromosome Information Service* **57**: 13–14.

Saitoh, K., Kumagai, Y. and Takahashi, M. (1992) Spermatocyte chromosomes of *Mycalepsis gotama nanda Frahstorfer*, 1908 (Lepidoptera: Satyridae), *Bull. Biogeogr. Soc. Jpn.* **47**: 117–119.

Seiler, J. (1914) Das Verhalten der Geschlechtschromosomen bei Lepidopteren Nebst einem Beitrag zur Kenntnis der Eireifung, *Arch. Zellforsch.* **13**: 159–269.

Suomalainen, E. (1953) The kinetochore and the bivalent structure in Lepidoptera, *Hereditas. (Lund.)* **39**: 88–96.

Suomalainen, E. (1965) On the chromosomes of the geometrid moth genus Cidaria, *Chromosoma (Berl.)* **16**: 166–184.

Suomalainen, E. (1969) On the sex chromosome trivalent in some Lepidoptera females, *Chromosoma (Berl.)* **28**: 298–309.

Suomalainen, E. (1971) Unequal sex chromosomes in a moth, *Lozotaenia forsterana* F. (Lepidoptera: Tortricidae), *Hereditas* **68**: 313–316.

Traut, W. (1986) A genetic linkage study of W-chromosome-autosome fusions, breakage and kinetic organization of chromosomes in *Ephestia* (Lepidoptera), *Genetica* **69**: 69–79.

Traut, W. and Rathjens, B. (1973) W-Chromosomes in Lepidoptera, *Genetics* **74**: s279.
Traut, W., Weith, A. and Traut, G. (1986) Structural mutants of the W chromosome in *Ephestia* (Insecta: Lepidoptera), *Genetica* **70(1)**: 69–79.
White, N. J. D. (1957) Cytogenetics and systematics entomology, *Ann. Rev. Entomol* **2**: 71–90.
White, N. J. D. (1973) *Animal Cytology and Evolution*, 3rd edition. Cambridge University Press.

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Evaluation of Toxicity of *Acorus calamus* L. Extracts to Various Stages of *Bactrocera cucurbitae* Coq.

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ABSTRACT. Toxicity of *A. calamus* extracts to various stages of *B. cucurbitae* were evaluated in a laboratory study. The various stages were treated with the extracts and mortalities determined after required intervals of time. The mortality values were subjected to Probit Analysis to work out the LC_{50} values. The aqueous extracts were not found to be toxic to any stage, upto 10% concentration. The solvent (methanol) extract was found to be 0.03% for eggs and 0.07% for adults. LT_{50} values were also calculated for a range of concentrations. © 2000 Association for Advancement of Entomology

KEYWORDS: *Acorus calamus*, *Bactrocera cucurbitae*, toxicity ovicidal action, LC_{50}, LT_{50}.

INTRODUCTION

One of the most common and destructive pests of cucurbits is the melon fly, *B. cucurbitae*. It is also one of the most difficult to control, and newer methods are always sought to manage its menace. Several plant species have been notes for their insecticidal effects and one among them, *viz.*, *A. calamus* is known to posses remarkable biological activity. Toxic effects of calamus extracts were observed on several field crop pests like *Spodoptera litura* (Sharma *et al.*, 1990) and (Prasad *et al.*, 1993) *Bagrada cruciferarum* (Verma and Pandey, 1981) *Athalia proxima* (Pandey *et al.*, 1979) etc and also storage pests. Laboratory experiments were carried out to evaluate the toxicity of *A. calamus* extracts to various stages of *B. cucurbitae*.

MATERIALS AND METHODS

Various stages of *B. cucurbitae* were obtained from a laboratory culture to ensure uniformity in age, size, sex, food and exposure to the environment.

Extracts of rhizomes of *A. calamus* were prepared by cold steeping method (Teotia and Pandey, 1979) using water and organic solvents. 10% stock solutions were first prepared and further required dilutions were made using the solvents, *viz.*, methanol.

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TABLE 1. Concentrations of *A. calamus* extracts tested for toxicity against various stages of *B. cucurbitae*

Stage	Aqueous extract (%)	Solvent extract (%)
Eggs	0.1	0.08
	0.5	0.06
	1.0	0.04
	5.0	0.02
	10.0	0.01
Larvae	0.1	0.1
	1.0	1.0
	5.0	5.0
	10.0	10.0
Pupae	0.1	0.1
	0.5	0.5
	1.0	1.0
	5.0	5.0
	10.0	10.0
Adults	0.1	0.1
	0.5	0.08
	1.0	0.06
	5.0	0.04
	10.0	0.02

Toxicity of *A. calamus* extracts to different life stages of *B. cucurbitae* was assessed by treating the target stages with the extracts and determining the mortality after required intervals of time. Concentrations of extracts tested against various stages are presented in Table 1. Six concentrations were tried with three replications for each treatment. Ten files were used for each replication. For untreated control, water or solvent was used.

To test ovicidal action, first the eggs were carefully extricated from the fruit pieces provided with the mated females for oviposition. The eggs were dipped in the extract for one minute and then removed using a fine camel hair brush onto a slice of fresh pumpkin fruit in a petri dish.

The eggs thus treated with different concentrations of the extracts were placed in separate Petri dishes for hatching overnight. The number of eggs hatched was determined, by counting the number of larvae emerged with in 24 hours. Eggs which did not hatch within 24 hours were taken as dead.

To test toxicity to larvae and pupae, ten numbers of each stage were collected per replication and were gently picked up and placed in a glass dish containing one ml. of the extract. They were kept in the extract for one minute and then removed to clean dry Petri dishes and kept for observation. Larvae were provided with pieces of fresh

pumpkin fruit to feed after the exposure. Larval mortality was recorded after 24 hours and pupal mortality based on eclosions.

To test toxicity to adults, two methods of application were used, namely topical application and residue film technique.

Topical application

The files were collected in a glass tube and kept tin the freezer compartment of a refrigerator for 10 minutes for temporary immobilization, as the flies are very active. One ml of the extract was taken in an atomizer and sprayed directly onto the immobilized flies after which they were released into glass chimneys and provided with sugar to feed.

Residue film technique

In this method, Petri dishes of 10 cm diameter were used to confine the flies. One ml of the extract was poured and smeared inside the upper lid and lower base of each Petri dish set. They were swirled to give a uniform coating of the extract on the inside and then dried under an electric fan. The flies were released in each dish set along with sugar to feed. Observations on mortality were taken at regular intervals. For all stages, the mortality values were converted to percentage mortality and the data were subjected to Probit Analysis according to Finney (1971).

RESULTS AND DISCUSSION

Ovicidal action

Concentrations tested and percentage egg mortalities are shown in Table 2. The aqueous extracts did not have ovicidal action.

Solvent extract concentrations which showed egg mortality in the range 70–30% were selected to calculate the LC_{50} to eggs. The LC_{50} of methanol extract of *A. calamus* to eggs of *B. cucurbitae* was found to be 0.03% (Table 3).

Toxicity to larvae & pupae

Mean percentage mortalities of larvae and pupae at various concentrations are shown in Table 4. The extracts did not affect both larvae and pupae upto 10% concentration which showed that there was no apparent contact toxicity of the *Acorus* principles on these stages of the insect.

Toxicity to adults

Concentrations of extracts and their corresponding percentage mortalities in topical spray and residue film technique are presented in Tables 5 & 6 respectively.

Topical spray

Aqueous extracts were not toxic. In case of methanol extract, the solvent itself was found to be toxic, so the solvent was evaporated and the extract diluted with water. But this was also not toxic (Table 5).

TABLE 2. Mean percentage mortality of eggs of *B. cucurbitae* treated with graded doses of *A. calamus* extracts

Treatment	Dose (%)	Mean percentage mortality*
Aqueous extract	1.10	0
	0.50	0
	1.00	0
	5.00	0
	10.00	0
	Control	0
Solvent extract	0.08	96.67
	0.06	90.00
	0.04	70.00
	0.02	36.64
	0.01	6.67
	Control	0.00

* Mortality observed after 24 hours.

TABLE 3. Probit Analysis of egg mortality

Slope (b)	−540.48
Intercept (a)	154.94
Standard Error of mean	0.035
Chi-square (Heterogeneity)	−101.58 (NS)
D.F	3
Log LC_{50}	−1.58
LC_{50}	0.03
Regression equation	$Y = 10.42 + 3.42x$
Fiducial limites	0.03 − 44.66

Residue film technique

Aqueous extract was not toxic in this case also. However solvent extracts showed varying degrees of mortality. Concentrations showing toxicity in the range 70–30% were selected and LC_{50} was calculated. LC_{50} was found to be 0.07% (Table 7).

LT_{50} values were also calculated for a range of concentrations by observing mortalities over different intervals of time (Table 8).

Toxicity of *A. calamus* to various stages of insects has been previously reported. Ovicidal action is by destruction of the embryo (Chander and Ahmed, 1986). Larvae were found to escape the toxic effect probably because they were released back into the fruits where th toxic material was lost by their tunnelling activity. The pupae also resisted the toxic effect owing to their tough covering. In case of adults, topical spraying was not found to be effective. However residue film technique showed some zomes are not toxic to *B. cucurbitae* owing to little or no contact action or penetration

TABLE 4. Mean percentage mortality of larvae and pupae of *B. cucurbitae* treated with graded doses of *A. calamus* extracts

Treatment	Dose (%)	Mean percentage mortality	
		Larvae	Pupae
Aqueous extract	0.10	0	0
"	0.50	0	0
"	1.00	0	0
"	5.00	0	0
"	10.00	0	0
"	Control	0	0
Solvent extract	0.10	0	0
"	0.50	0	0
"	1.00	0	0
"	5.00	0	0
"	10.00	0	0
"	Control	0	0

TABLE 5. Mean percentage mortality of *B. cucurbitae* adults treated with graded doses of *A. calamus* extracts by topical application

Treatment	Dose (%)	Mean percentage mortality*
Aqueous extract	0.10	0
"	0.50	0
"	1.00	0
"	5.00	0
"	10.00	0
"	Control	0
Methanol extract (redissolved in acetone)	0.10	100
	0.50	100
"	1.00	100
"	5.00	100
"	10.00	100
"	Control	100
Methanol extract (diluted with water)	0.10	0
	0.50	0
"	1.00	0
"	5.00	0
"	10.00	0
"	Control	0

* Mortality observed after 24 hours.

TABLE 6. Mean percentage mortality of *B. cucurbitae* adults treated with graded doses of *A. calamus* extracts by residue film technique

Treatment	Dose (%)	Mean percentage mortality*
Aqueous extract	0.10	0
	0.50	0
	1.00	0
	5.00	0
	10.00	0
	Control	0
Methanol extract	0.10	100.0
	0.08	73.33
	0.06	23.33
	0.04	6.67
	0.02	0.00
	Control	0

* Mortality observed after 24 hours.

TABLE 7. Probit Analysis of Adult Mortality

Slope (b)	:	−313.2
Intercept (a)	:	72.77
Standard Error of mean	:	0.014
Chi-square (Heterogeneity)	:	−6.67 (NS)
D.F	:	3
Log LC_{50}	:	−1.17
LC_{50}	:	0.07
Regression equation	:	$Y = 16.79 + 10.03x$
Fiducial Limits	:	0.07 − 15.74

TABLE 8. LT_{50} values of various concentrations of *A. calamus* extracts on adults of *B. cucurbitae*

Concentration (%)	Heterogeneity (χ^2)	Regression equation	LT_{50} (hours)	Fiducial limits	
10.0	0.039	$Y = 4.54 + 11.54x$	1.10	1.16	−0.96
5.0	−51.77	$Y = 2.3 + 5.2x$	3.31	3.65	−2.99
1.0	4.00	$Y = -5.49 + 9.65x$	12.30	13.03	−11.61
0.1	7.34	$Y = 11.05 + 12.73x$	20.89	21.98	−19.86
0.07	1.22	$Y = 5.56 + 7.69x$	23.44	26.24	−20.94
0.025	−9.34	$Y = -18.36 + 13.05x$	61.66	102.32	−37.15

Y = Probit kill, x = Log LT_{50}.

of the toxic principle. However solvent extracts show varying degrees of toxicity to eggs and adults. But the treatment of both these stages was done in confined conditions. A technique for field application is yet to be perfected.

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REFERENCES

Chander, H. and Ahmed, S. M. (1986) Efficacy of oils from medicinal plants as protectants of green gram against the pulse beetle, *Callosobruchus chinensis*, *Entomon* **11(1)**: 21–28.

Finney, D. J. (1971) *Probit Analysis*, 3rd edition. S. Chand and Co. Ltd.: New Delhi, 333.

Pandey, N. D., Sudhakar, T. R., Tewari, G. C. and Pandey, U. K. (1979) Evaluation of some botanical antifeedants under field conditions for the control of *Athalia proxima* Kulg, *Indian J. Ent.* **41(2)**: 107–109.

Prasad, V. D., Jayaraj, S., Rabindra, R. J. and Reddy, G. P. V. (1993) Studies on the Interaction of Certain Botanicals and NPV Against Tobacco Caterpillar *Spodoptera litura* F, *Botanical pesticides in integrated pest management*. Indian Society of Tobacco Science: Rajahmundry, India, 190–196.

Sharma, R. N., Deshpande, P. S. G. and Joseph, M. (1990) Evaluation of natural essential oils as antifeedants against *Spondoptera litura* (Fab.), *Indian Perfumer* **34(30)**: 196–198.

Teotia, T. P. S. and Pandey, G. P. (1979) Insecticidal properties of rhizomes of sweet flag, *Acorus calamus* against rice weevil, *Sitophilus oryzae* L., *Indian J. Ent.* **41(1)**: 91–94.

Verma, G. S. and Pandey, U. K. (1981) Studies on the effect of *Acorus calamus*, *Cimicifuga foetida* and *Gynandropsis gynandra* extract against insect pests of cruciferous vegetables—painted bug–*Bagrada cruciferarum* Kirk. (Hemiptera: Pentatomidae), *Z. Angew. Zoo* **68(1)**: 109–113.

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Evaluation of Some Insecticides and Natural Products Against Coffee White Stem Borer, *Xylotrechus quadripes* Chevr. (Coleoptera: Cerambycidae)

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ABSTRACT: Insecticides *viz.*, endosulfan 35 EC and chlorpyriphos 20 EC; botanical insecticides *viz.*, nimbecidine and neem sludge and other products such as cashew oil, lime and bitumen were tested against *X. quadripes* in comparison with conventional insecticide, Lindane 20 EC in the laboratory. Nimbecidine and lindane combinations were also screened against the pest. Efficacy of botanical insecticides, lime and bitumen varied from 22 to 35 days while that of chlorphyriphos, lindane and nimbecidine combinations and endosulfan was 77 to 83 days.
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KEYWORDS: *Xylotrechus quadripes*, *Coffea arabica*, insecticides, screening.

Coffee white stem borer, *Xylotrechus quadripes* Chevr. is the most serious pest of arabica coffee, *Coffea arabica* L. The borer has been reported in India as early as 1897 (Subramaniam, 1934). The pest also occurs in China, Sri Lanka, Vietnam and Thailand (Le Pelley, 1968) and several countries of South Africa (Schoeman, 1990). The stem borer causes substantial economic loss per annum to Indian coffee industry, as pest infested productive plants have to be uprooted twice a year. BHC 50 WP at 1% and its gamma isomer, Lindane 20 EC at 0.13% are recommended for the control of *X. quadripes* (Anonymous, 1997). Regularly used BHC 50 WP against *X. quadripes* has been banned and envisaging the forbid of Lindane 20 EC in near future in India, finding of alternative effective environment friendly chemicals for the control of the stem borer is highly essential. Therefore, a preliminary investigation on effectiveness of various insecticides and natural products against *X. quadripes* in the laboratory was conducted to find out the alternate and effective insecticides.

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TABLE 1. Results of the laboratory screening of various products against *Xylotrechus quadripes*

Sl. No.	Products (% Concentration)	Oviposition deterrence (in days)	Efficacy/Cent per cent larval mortality (in days)
1	Bitumen*	6.0[a]	29[a]
2	Cashew oil (0.6)	1.5[a]	25[a]
3	Cashew oil (1)	1.0[a]	29[a]
4	Cashew oil (1.5)	1.5[a]	29[a]
5	Cashew oil (5)	1.5[a]	35[a]
6	Lime (5)	2.0[a]	35[a]
7	Lime (10)	2.0[a]	35[a]
8	Neem sludge*	9.0[a]	32[a]
9	Nembecidine (2)	2.0[a]	22[a]
10	Nembecidine (5)	2.0[a]	22[a]
11	Nembecidine (2) + Lindane 20 EC (0.06)	52.0[b]	78[b]
12	Nembecidine (2) + Lindane 20 EC (0.13)	61.0[c]	82[b]
13	Chlorpyrifos 20 EC (0.06)	59.0[b]	77[b]
14	Endosulfan 35 EC (0.06)	63.0[c d]	83[b]
15	Lindane 20 EC (0.13)	69.0[d]	83[b]
	CD 0.05	8.72	36.00
	CD 0.01	11.74	48.47

*Undiluted form; Results with in a column followed by the same letter are not significantly different.

Fresh arabica cut stems (15 cm length and 8–10 cm diameter) were sprayed with various insecticide solutions and natural products using hand sprayer. The stems treated with Lindane 20 EC were kept as a standard check. The teated stems were kept individually in a plastic container (18 cm height and 26 cm diameter) and a gravid *X. quadripes* beetle was allowed for egg laying. Dead beetles in the containers were immediately replaced with the new ones. Each treatment consisted three replications and there were three stems per replication. Observations on the oviposition deterrence and mortality of hatched out larvae were made daily after treatment to assess the efficacy of different products. Recordings were made only until cent per cent mortality of hatched out larvae was noticed with different products after treatment, as even tunneling of single larva into the stem will affect the plant.

Efficacy of various products screened against *X. quadripes* in the laboratory is given in Table 1. However, the beetles deposited eggs on the stems treated with cashew oil,

lime and nimbecidine in 1–2 days and that on the bitumen and neem sludge treated stems in 6 and 9 days respectively after treatment, cent per cent larval mortality could be seen for 22–35 days. It was noticed that beetles laid eggs on the stems treated with chlorpyriphos, lindane + nimbecidine combinations, endosulfan and lindane after 52 to 69 days of treatment (Table 1). Though the oviposition deterrence in lindane (0.06%) + nimbecidine (2%) mixture is significantly ($P > 0.05$) lower than that of lindane (0.13%) + nimbecidine (2%), endosulfan and lindane, it is significantly ($P > 0.01$) higher than that of botanical and other natural products.

Effectiveness of neem formulations and other natural products is not significantly ($P < 0.05$) different from each other. Cent per cent larval mortality could be seen up to 77 to 83 days in treatments such as chlorpyriphos, endosulfan, nimbecidine + lindane combinations. Efficacy of these insecticides is not significantly different ($P < 0.05$) from that of conventional insecticide Lindane 20 EC. Effectiveness of the botanical insecticides and natural products is significantly lower ($P > 0.05$) than that of all the tested organic insecticides including neem + lindane mixtures. It is obvious from the study that mixing of neem formulations with lindane has no added advantage over the application of lindane alone and, mixing of these chemicals is found to cause only additional expenditure.

Endosulfan 35 EC at 0.06% is an insecticide of choice for the control of coffee berry borer, *Hypothenemus hampei* (Ferrari) (Anonymous, 1997). As efficacy of endosulfan was on par with that of lindane, a single spray of endosulfan could be tried for the control of both *X. quadripes* and *H. hampei* during October–December, when the incidence of these borers overlaps. Because botanical insecticides and natural products were found to give protection for a few days even in the laboratory, their efficiency in the control of long prevailing *X. quadripes* has to be verified in the field. Since efficacy of various organic insecticides is on par with that of lindane, these chemicals can be tested in the field to assess their usefulness in the control of *X. quadripes*.

REFERENCES

Anonymous (1997) *Coffee Guide*, Central Coffee Research Institute, Coffee Research Station: Chikmagalur District, Karnataka, India, 179.

Le Pelley, R. H. (1968) *Pest of Coffee*, Longmans, Green & Co. Ltd: London, 590.

Schoeman, P. S. (1990) Control of the coffee white stem borer, *Inlichtingsbulletin Navorsings-instituut-vir-Sitrus-en Subtropiese Vrugte* **220**: 3–4.

Subramaniam, T. V. (1934) The coffee white stem borer. Department of Agriculture, Mysore State, *Entomological Series, Bulletin* **11**: 18.

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Effect of L-Glycine on Growth of Silkworm *Bombyx mori* L.

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ABSTRACT: Amino acid L-Glycine (2 and 3%) was supplemented to two races of silkworm *Bombyx mori* L. Pure Mysore and NB_4D_2 through leaf in four different schedules. It was observed that glycine supplementation influenced majority of the growth traits. Fifth instar larval duration was not influenced significantly in both Pure Mysore and NB_4D_2. However, mature larval weight, silk gland weight and spinning per cent were significantly higher among the larvae fed with Glycine supplemented leaves than those with no supplementation. © 1999 Association for Advancement of Entomology

KEYWORDS: Glycine, *Bombyx mori* L., mature larval weight, silkgland weight, spinning per cent.

The nutritional status of mulberry leaf influences the silk productivity both quantitatively and qualitatively. This factor in addition to influencing the growth and development of silkworm also has a role to play for disease epizootics to occur and finally reducing the silk production (Nagarajan and Radha, 1990). Among many ways of improving the potentiality of silkworm for enhanced productivity, proper nutritional management appears to be the most important method.

Many earlier workers have established and suggested the supplementation of amino acids through mulberry leaves for the improvement of performance of silkworms in their economic concern. Legay (1958) has reported that qualitative and quantitative sericultural output is dependant on nutritional status of mulberry leaves. Pant (1978) envisaged clearly that utilizing nutritional data for exploitation of beneficial insects like silkworm, has a great scope and stressed that the qualitative and quantitative aspects of silk yield could be directly increased through proper dietary management. Hence, proper care of silkworm through dietary management is an essential requisite to maximise sericultural output. Krishnappa (1987) established the superiority of glycine to other amino acids in improving economic parameters of silkworms. In order to ascertain the concentration of glycine, time and frequency of supplementation, the present study was undertaken.

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In this study two races NB_4D_2 and Pure Mysore were mass reared separately by adopting standard rearing practices (Benchamin and Nagaraj, 1987). The worms just out of fourth moult were used. The treatments were replicated thrice with 100 worms each.

L-Glycine at 2 (T_1) and 3 per cent (T_2) in tap water was supplemented in four schedules *viz.*,

01 Once a day in V instar (S_1)
02 Twice a day in V instar (S_2)
03 Once a day from second to fifth day of V instar (S_3)
04 Twice a day from second to fifth day of V instar (S_4).

Three controls were maintained out of which, two were tap water treated controls *viz.*, water treatment once a day (W_1) and twice a day (W_2), to judge the impact of water in glycine solution. The plain control consisted of worms fed with untreated leaves (C).

L-Glycine solution (10 ml) of required concentration was uniformly smeared to 50 g of mulberry leaf by mixing and dried in shade for removing excess moisture, then the same was fed to 100 worms of one replication. When the supplementation was once a day it was preferably followed during first feed of the day and when ever treatment was twice a day, it was followed in the first and last feed of that day to ensure that the entire treated leaves were consumed by the larvae. Similar procedure was followed for water supplementation also.

The study has clearly shown that the growth parameters in silkworm races Pure Mysore (PM) and NB_4D_2 were positively influenced due to glycine fortification. The fifth instar duration did not vary significantly (Table 1).

The mature larval weight increased significantly due to glycine supplementation (Table 1). In PM, 3 per cent glycine registered its superiority by yielding higher larval weight (19.70 to 20.42 g/10 worms) compared to 2 per cent (18.37 to 20.08 g) and control (16.38 g), while in NB_4D_2 it ranged from 39.21 to 42.10 g (at 2%) and 37.29 to 40.82 g (at 3%), which was a significant improvement over control (35.51 g).

Majority of glycine treatments in PM showed a significant improvement in silkglands weight over control (0.393 g) (Table 1). In general glycine supplemented throughout fifth instar evoked better enhancement of silkglands weight (0.454 to 0.483 g). Glycine 3 per cent was better (0.425 to 0.483) than 2 per cent (0.415 to 0.458 g). In NB_4D_2 all glycine treatments (1.32 to 1.187 g) showed significantly higher silkglands weight compared to control (0.821 g).

Rate of spinning was significantly influenced in both PM and NB_4D_2 by glycine supplementation. Supplementation twice a day caused significant rise in spinning of PM (93.00 to 95.33%) compared to once a day application (91.33 to 91.67%) and all the glycine treatments were significantly superior to control (84.00%). Even in NB_4D_2 spinning was significantly higher with glycine (89.70 to 91.65%) compared to control (81.42%).

TABLE 1 Effect of glycine supplementation of mulberry leaf on growth of PM and NB_4D_2 breeds of silkworm *Bombyx mori* L.

Treatments	V instar larval duration (h) PM	V instar larval duration (h) NB_4D_2	Ten mature larval weight (g) PM	Ten mature larval weight (g) NB_4D_2	Weight of silk glands (g) PM	Weight of silk glands (g) NB_4D_2	Spinning% PM		Spinning% NB_4D_2	
T_1S_1 [illegible] Glycine once a day in V instar	224.7	[illegible]	18.42	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	(89.70)	[illegible] 417
T_1S_2 [illegible] Glycine twice a day in V instar	22[illegible] 0	[illegible]	18.37	[illegible]	[illegible]	1.170	(9[illegible])	[illegible] 22	(90.60)	[illegible]
T_1S_3 [illegible] Glycine once a day from 2nd to 5th day of V instar	222.40	[illegible]	18.57	[illegible]	[illegible]	[illegible]	(90.[illegible])	[illegible] 47	8[illegible] 70	[illegible] 417
T_1S_4 [illegible] Glycine twice a day from 2nd to 5th day of V instar	[illegible]	[illegible]	[illegible]	[illegible] 85	[illegible]	[illegible]	[illegible]	[illegible]	(89.78	[illegible]
T_2S_1 [illegible] Glycine once a day in V instar	224.30	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	(89.88	[illegible]
T_2S_2 [illegible] Glycine twice a day in V instar	225.30	[illegible]	[illegible]	40.02	0.48[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T_2S_3 [illegible] Glycine once a day from 2nd to 5th day of V instar	224.30	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] 2.922	(90.48	[illegible]
T_2S_4 3% Glycine twice a day from 2nd to 5th day of V instar	224.70	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
W[illegible] Water treatment once a day	22[illegible] 30	19[illegible] 0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	65.668	(79.8[illegible]	63 [illegible]
W[illegible] Water treatment twice a day	225.00	195.6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	65.409	(7[illegible] 11	6[illegible] 8[illegible]
[illegible] Control	22[illegible] 30	19[illegible] 0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	66.496	(81.45)	6[illegible] 19
F test	NS	NS	*	*	*	*	*		*	
SEm ±	[illegible] 3257	1.4597	0 [illegible] 018	[illegible]	0.008[illegible]	0.0[illegible]	[illegible] 4382		1.44[illegible]	
CD at 5%	[illegible] 9110	4.3061	0 [illegible] 002	[illegible]	0.0[illegible]	0.04[illegible]	[illegible] 242[illegible]		[illegible]	
CV (%)	1.0226	0.3010	0[illegible] 0	[illegible]	3.458[illegible]	2.6[illegible]	[illegible] 4446		[illegible] 30	

* Significantly different at 5 per cent. [illegible] Figures in parentheses [illegible] NS - Nonsignificant.

In the present study larval duration did not differ significantly due to L-glycine supplementation. In contrary Krishnappa (1987) observed reduction in fifth instar and total larval duration in Pure Mysore and NB_4D_2 due to supplementation of L-alanine, glycine, L-phenyl alanine and L-tryptophan, with more pronounced effect with glycine. Reduced larval duration also resulted when leaves dipped in rain water were fed to silkworm during winter (Thangavelu and Bania, 1990).

Twice a day supplementation of glycine recorded higher larval weight compared to daily once. The observations are in close confirmity with those of Murthy and Sreenivasaya (1953), Sharada *et al.* (1956), Sengupta *et al.* (1972), Davis (1978), Shridhar and Radha (1986) and Moustafa and Karaksy (1988) who also observed significant increase in larval weight due to glycine fortification. However, Sharada and Bhat (1956) did not notice considerable effect of chloromycetin and glycine on larval weight. Ito and Arai (1967) opined that addition of non-essential amino acids improved larval growth and development and the effect was increased by increasing dietary levels of non-essential amino acids. Jadhav and Kallapur (1989) surmised that both fat body and integument of silkworms synthesised proteins from the supplemented amino acids and accumulation of the same might increase the larval weight.

Glycine 3 per cent supplemented twice a day from second to fifth day of fifth instar was the best. Eid *et al.* (1988) also observed increased silkgland weight due to excess amino acids supplemented to eri silkworm through castor leaf and opined that amino acid supplementation increased the enzymatic activity of silkglands which inturn increased silk protein synthesis, which might have contributed for the increased weight of silkglands. Crude protein content of silkglands was elevated due to alanine, glycine and serine supplementation which inturn contributed for increment in weight of silkgland (Moustafa and Karaksy, 1988).

Present observations on rate of spinning which was superior when Glycine 3 per cent fortified twice a day, agree with the findings of Sengupta *et al.* (1972) who observed increased E.R.R with increased glycine concentration. According to Shridhar and Radha (1986) also, glycine significantly increased the per cent recovery of cocoons in KA × NB_{18} hybrid. Krishnappa (1987) too reported that glycine supplementation significantly increased survival in Pure Mysore and NB_{18}.

It can be thus inferred that supplementing the mulberry leaves with glycine (3%) twice a day from second to fifth day of fifth instar has been found to improve the growth of mulberry silkworm.

REFERENCES

Benchamin, K. V. and Nagaraj, C. S. (1987) Silkworm rearing techniques, In: *Appropriate Sericulture Techniques*, Jolly, M. S. (Editor), Central Sericultural Research and Training Institute: Mysore, 63–106.

Davis, G. R. F. (1978) Growth response of larvae of *Tenebrio molitor* to concentrations of dietary amino acids, *J. Stored Products Res.* **14**: 69–71.

Eid, M. A. A., El-Nakkady, A. N. and Salem, M. A. (1988) Effect of supplementary amino acids on silk secretion by larvae of *Philosamia ricini* (Boisd.), *Indian J. Seric.* **28**: 224–232.

Ito, T. and Arai, N. (1967) Nutritive effect of alanine, cysteine, glycine, serine and tyrosine on silkworm, *Bombyx mori* L., *Insect Physiol.* **13**: 1813–1824.

Jadhav and Kallapur V. L. (1989) Contribution of tissue proteins to the cocoon shell in the 5th instar silkworm, *Bombyx mori* L., *Entomon* **14**: 21–24.

Krishnappa, J. B. (1987) Influence of amino acids supplementation on growth and development of mulberry silkworm, *Bombyx mori* L. M. Sc. (Seri.) Thesis, University of Agricultural Sciences, Bangalore, 128.

Legay, J. M. (1958) Recent advances in silkworm nutrition, *Ann. Rev. Entomol* **3**: 75–86.

Moustafa, S. M. and Karaksy, E. L. (1988) The effect of certain amino acids on silkworm *Bombyx mori* L., *Agri. Res. Rev.* **66**: 105–109.

Murthy, M. R. V. and Sreenivasaya, M. (1953) Effect of antibiotics on the growth of silkworm *Bombyx mori* L., *Nature* **172**: 684–685.

Nagarajan, P. and Radha, N. V. (1990) Supplementation of amino acids through mulberry leaf for increased silk production, *Indian Silk* **20(4)**: 21–22.

Pant, N. C. (1978) Insect nutrition in economic entomology, In: *Insect Physiology and Anatomy*, Pant, N. C. and Swaraj Ghai, (Editors), I.C.A.R.: New Delhi, 213–227.

Sengupta, K., Singh, B. D. and Mustafa, J. C. (1972) Nutrition of silkworm *Bombyx mori* L. Studies on the enrichment of mulberry leaf with various sugars, proteins, amino acids and vitamins for vigorous growth of worm and increased cocoon crop production, *Indian J. Seric.* **11**: 11–19.

Sharada, K. and Bhat, J. V. (1956) Effect of chloromycetin and glycine on the growth and reproduction of silkworm *Bombyx mori* L., *J. Indian Inst. Sci.* **38**: 136–147.

Sharada, K., Leena, T., Shroff, Shyamala M. B., Iyengar K. S. and Bhat J. V. (1956) Large scale experiments on the effect of chloromycetin and glycine in the nutrition of silkworm, *Bombyx mori* L., *J. Indian Inst. Sci.* **38**: 136–147.

Shridhar, P. and Radha, N. V. (1986) Effect of supplementing glycine to the feed of silkworm, *Nat. Semi. Prob. Prosp. Seric. in India*, Vellore, Tamil Nadu. 18.

Thangavelu, K. and Bania, H. R. (1990) Preliminary investigation on the effects of minerals in the rain water on the growth and reproduction of silkworm*Bombyx mori* L., *Indian J. Seric.* **29**: 37–43.

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Infestation of Pentatomid Bugs on *Pongamia glabra* W. in the B. R. Project Area of Western Ghats, Karnataka

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ABSTRACT: A study on the status of infestation of the forest plants, *Pongamia glabra (pinnata)* of the Western ghats region of B. R. Project, was undertaken during the period July, 1997 to October 1998 respectively. It was revealed that about 17.7 to 18.88% and 20.99 to 37.6% of the trees were infested by the pentatomid bugs during the years 1997 and 1998 respectively. It was also observed that the host plants having infestation for consecutive 2–3 years suffered severe damage and it proved fatal to the trees. Since the host tree is an important medicinal plant, all parts of the plant *viz.*, bark, leaves, roots and fruits are used in the ayurvedic medicine as an important ingredient. The oil prepared by the seeds of the plant is used as an alternate to the fuel, diesel in the rural Karnataka and the leaves are used in the agricultural field as manure. Protection of such valuable trees from the Pentatomid bug, *Cyclopelta siccifolia* infestation is felt urgently needed. © 2000 Association for Advancement of Entomology

KEYWORDS: Pentatomid bugs, *Cyclopelta siccifolia*, *Pongamia glabra*, and Karanja.

INTRODUCTION

About 10,000 years ago humans began to settle and cultivate crops. This required that the diverse natural vegetation was replaced by few species of crops. The loss of biological diversity of natural ecosystems also destroyed a major part of fauna, while enabling a few agro-ecosystem to flourish. Some pests and weeds survived and multiplied in the new agro-ecosystems. These species began to compete with man to food, feed, forage, fiber etc., and we thus describe them as pests (Piemental, 1997).

The ecosystem of Western Ghats is the storehouse of vast flora and fauna in which many of them are new to science. The members of the family Pentatomidae are phytophagus hemipteran bugs, infest a variety of vegetable crops and forest trees (Shashikumar *et al.*, 1996). The members of Pentatomidae belonging to the genera

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TABLE 1. Total number of infested and noninfested host plants during the year 1997

Zones	Noninfested trees (a)	Infested trees (b)	Percentage
South	302	65	a. 82.2 b. 17.7
East	125	29	a. 81.1 b. 18.8

Antestiopsis, *Bagrada*, *Calidea*, *Diploxys*, *Nazare* and *Obalus* have been studied as agriculture pests (Hill, 1993).

The nymph and adult stages of *Cyclopelta siccifolia* infest forest trees namely *Pongamia glabra* and *Erythrina indica*. The life history of *Cyclopelta siccifolia* comprises 7 nymphal instars, all stages are phytophagus (Hosetti *et al.*, 1998). In the study area most of the *Pongamia glabra* were infested by *Cyclopelta siccifolia*. Usually these trees are planted as avenue trees. Pongamia species is a medium sized, growing up to the height of about 18 meters, drought and saline resistant belongs to the family *Leguminosae*. It is commonly called as Karanja (Hindi), flowers are small, contain Kaempferol and waxes, hence medically important. Leaves are used as fodder and as green manure. Roots contain four furoflavines, namely, Karanjin, Pongapin, Pinnatin and Gamatin (Tanka *et al.*, 1992). Fruit kernel (about 95%) contain oil used in soap, grease, lubricant and leather tanning. Application in skin disease in pharmaceuticals and as disinfectants is also recorded. Oil can be refined by cold extraction with alcohol and subsequent refining and bleaching to remove the smell and colour and then it may be used for edible purpose. The Kernel extract is also having some antiviral property (Hosetti *et al.*, 1997). Very recently in some places of South Karnataka this oil is used to run generators and pump sets to lift water and to counteract the scarcity and high price of diesel and electricity. The present work attempts to report the status of infestation of Pentatomid bug, *Cyclopelta siccifolia* on *Pongamia glabra* trees in Western Ghats region of Karnataka state.

STUDY AREA

To study the impact of infestation of Pentatomid bugs *Cyclopelta siccifolia* on *Pongamia glabra* of B. R. Project area near the University campus was selected. To evaluate the infestation by Pentatomid bugs the study area was conveniently divided in to 2 zones. The University campus was considered as central point and two zones from the centre point that is up to 5 km South and East were selected. In the rest of the areas there were no trees recorded.

TABLE 2. Total number of colonies and individual bugs found on *Pongamia glabra* July–October 1997

Zones	No. of infested trees	No. of colonies	Total No. of Nymphs	Total No. of adults	Total population
South	65	1998	26,50,36	9861	27,48,97
East	29	1488	25,65,81	9417	26,59,98

TABLE 3. Status of infestation during July–October 1997

Zones	Free from infestation (a)	Mild (b)	Severe (c)	Lethal (d)	Dead (e)	Total %
South	302	50	10	5	nil	a. 82.2 b. 13.6 c. 2.7 d. 1.3
East	125	19	7	3	nil	a. 81 b. 12.3 c. 4.5 d. 1.9

METHODS

The insect colonies below the age of IV instar nymphs were enumerated by direct count method, where as individual organisms were counted by sweeping method (Anonymous, 1990).

RESULTS AND DISCUSSION

The *Pongamia* species are widely planted as avenue trees in the University campus, Engineering quarters, B. R. Project area and road sides. Additionally these trees are available as forest plants in the foot hills of Western Ghats adjacent to our study area. The infestation problem of the Pentatomid bug, *Cyclopelta siccifolia* on the *Pongamia glabra (Pinnata)* trees were studied for a period of 2 years (July 1997 to October 1998). The rate of infestation during 1997 (Tables 1, 2 and 3) and for during 1998 (Tables 4, 5 and 6) were recorded. In 1997 a total of 94 infested trees were recorded. Out of these 65 trees found in the south zone, which comprises of 2,74,897 bugs. In East zone 1488 colonies infesting only 29 trees with a population of 2,65,998 bugs was recorded. In the following year 1998 the number of infested trees were comparatively more. As in the previous year a total of 65 trees infested by Pentatomid in the South zone harbored 2,10,632 bugs whereas in the East zone the number of infested trees increased to 47 trees with a population of 2,08,163 bugs.

The rate of infestation of Pongamia trees was not uniform, it varied from mild to lethal levels. The data printed in qTable 3 reveals that the rate of infestation in the year

TABLE 4. Total percentage of infested and noninfested trees during July–October 1998

Zones	Noninfested (a)	Infested (b)	Percentage
South	246	65	a. 79 b. 20.9
East	78	47	a. 62.4 b. 37.6

TABLE 5. Total number of colonies and individuals found on *Pongamia glabra* during July–October 1998

Zones	No. of infested trees	No. of colonies	Total Nymphs	Total adults	Total population
South	65	1061	20,00,61	10,571	21,06,32
East	47	772	19,80,81	10,081	20,81,63

TABLE 6. Status of infestation during July–October 1998

Zones	Non infested trees (a)	Mild (b)	Severe (c)	Lethal (d)	Dead (e)	Total %
South	246	47	13	3	2	a. 67 b. 10.3 c. 3.5 d. 0.8 e. 0.5
East	78	34	10	2	1	a. 67 b. 22.0 c. 6.4 d. 1.2 e. 0.6

Colonies-1–75 -Mild, 75–150-Severe, 150-Above-Lethal.

1997 was mild (13.6%), severe (2.7%), lethal (1.3%) and dead (nil) in South zone and in the range 12.3%, 4.5%, 1.9% in the East zone respectively. In the subsequent year the infestation rate at South zone mild (10.3%), severe (3.5%), lethal (0.8%) and dead (0.5%) and 22.0, 6.4, 1.2 and 0.6% was recorded in the East zone respectively. The data on status of infestation during the 2 seasons proved that 17.7 to 18.8% infestation in the year 1997 and 20.9 to 37.6% in 1998 was recorded respectively.

The laboratory study under progress in our Department and the literature published already (Hosetti *et al.*, 1998) revealed that these bugs are resistant to most of the

chemical pesticides. These are also resistant to most of the biological pests. It was observed that the adults were accepted by Myna birds. Another interesting observation made is that the nymphs of 6th and 7th instar were suffering from fungal diseases. The symptoms included that the nymphs become sluggish for one or two days, the body turns shining red on the 3rd and 4th day and died on day 5th. The dead animals were incubated under laboratory conditions for a period of one week and it was found that the entire body surface of moribund was covered by mycelial growth and the fungi responsible for the disease is yet to be identified.

These insects may be controlled either by destroying the eggs manually during July–August while breeding season is on or by using chemicals during nymphal stages soon after ecdysis. Since the host trees provide very important ingredients in the Ayurvedic medicine, in the preparation of green manure lubricating oil and as an alternative to diesel in the Karnataka state (seed oil), there is a need to protect these trees. Any tree infested by these bugs suffering severely for 2 to 3 years may lead to the death of the trees. As the trees are economically very beneficial to man kind, there is a urgent need to develop the pest control strategy for bugs.

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REFERENCES

Hosetti, B. B., Ujwal, P., Mohan, B. S. and Krishnamurthy, M. (1998) Biology of Pentatomid bug, *Cyclopelta siccifolia* West wood. *Indian J. Forestry* **21** (3): 232–237.

Hosetti, B. B., Belagali, S. L., Ujwal, P. and Mohan, B. S. (1997) Pentatomid bugs also serve, *Chemistry and Biology of Herbal medicine* 79–82.

Shashikumar, K. C., Hosetti, B. B., Jeba Kumar, J. P. P. and Madhyastha, M. N. (1996) Ecology of Pentatomid bug *Cyclopelta siccifolia* with reference to foraging behaviour, *Environment and Ecology* **14**: 828–833.

Anonymous (1990) *Workshop Manual on Insect plant interactions*, Published by The Director, Entomology Research Institute, Loyola College, Madras.

Tanka, T. M., Linuma, K., Yuvi, K. F. and Mizuno, M. (1992) Flavanoides in the root bark of *Pongamia pinnata*, *Phytochemistry* **31**: 995–998.

Hill, H. S. (1993) *Agricultural Insects Pests of Tropics and their Control*, 2nd edition. Cambridge University Press: New York.

Pimental, D. (1997) Ecological basis of Insect pest pathogen and weed problem. In: *Origin of Pest Parasite Disease and Weed Problems*, Cherret, J. M. and Sagar, G. R. (Editors). Backwell Scientific publishers: Oxford, 3–31.

Derle, N. R. 12th August 1999. Alternate to Diesel. Taranga Publishers Manipal, Karnataka. 61 & 68.

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Predatory Spider Behaviour in Rice Varieties Under Sodic Soil Conditions

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ABSTRACT. The relationship between the predatory behaviour of spiders and the rice varieties on which these harbour was studied at the Agricultural College and Research Institute, Tiruchirappalli. It was observed that the population of spider in descending order was *Argiops* (14.15) > *Tetragnatha* (7.75) > *Lycosa* (7.05). Among the varieties, TRY 1 harboured more web spinning spiders i.e. *Argiops* (16.8) and *Tetragnatha* (9.1) > *Lycosa* (5.4) in the experiment area while CO43 harboured less number of web spinners and the population per 25 m^2 was 11.5 and 6.4 respectively in these varieties. The non webbing spider i.e. wolf spider, Lycosa sp., was found to be more in CO43 (8.7) than TRY 1 (5.4), probably due to open niche of the former which favoured the spider. © 2000 Association for Advancement of Entomology

KEYWORDS: Spider, *Argiops*, *Tetragnatha*, *Lycosa*.

Spiders living in cultivated fields show relatively high population densities. Together with the carnivorous beetles they form the most abundant ground dwelling predators of insects in cereal fields. (Ganesh Kumar and Velusamy, 1996). Spiders are the predators of green leaf hopper, brown planthopper and yellow stem borer. Lycosids, the dominant group of spiders (Shivamurthappa, 1993) take an average 100 days to reach the adult stage (Gavarra and Raros, 1975; Samal and Misra, 1985) which is almost one cropping season. Hence the conservation of adult spiders is necessary. The present study aims at studying relationship between the predatory behaviour of spiders and the rice varieties on which they harbour.

A survey was conducted in the paddy fields of Agricultural College and Research Institute, Tiruchirappalli during the samba season of the year 1997–1998. Fortnightly observations were made on the population of various spider species *viz*., Orb weaver spider (*Argiopes* sp.), long jawed spider (*Tetragnatha* sp) and wolf spider (*Lycosa pseudoannulata*) on two rice varieities *viz*., TRY and CO43. Of the above spider sp. the former two sp. have the habit of web spinning. Hence, the number of webbings per 25 sq. meter (5 × 5 m square) was counted at 10 different locations of each rice variety, for the non web spinning spider *viz*., *Lycosa* sp., the no. of spider per 25 sq.

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TABLE 1. Spider population as affected by rice varieties in sodic soil condition

Rice Variety	Population per 25 sq. m. [±SE(0.05)]		
	Orb Weaver Spider	Long Jawed Spider	Wolf Spider
TRY–1	16.8(±1.25)	9.1(±2.40)	5.4(±0.53)
CO43	11.5(±1.19)	6.4(±1.75)	8.7(±1.28)
Mean	14.15	7.75	7.05

cm area was counted in each of the rice variety. The mean value of the population of different spider species in the two rice varieties was tabulated. They prey insect species trapped in the spider webs observed during the survey were collected, identified and their population was counted and tabulated.

The mean data on the population of spider species are presented in Table 1. It was observed that the population of spiders is descending order were *Argiops* (14.15) > *Tetragnatha* (7.75) > *Lycosa* (7.05). Among the varieties TRY 1 harboured more of web spinning spiders i.e. *Argiops* (16.8) and *Tetragnatha* (9.1) > *Lycosa* (5.4) in the experiment area. The rice variety CO43 harboured less number of web spinners and the respective population per 25 square metre was 11.5 and 6.4 respectively. The reason for the difference in web spinning population between varieties was attributed to the crop canopy. The varieity TRY 1 was observed to be more dense with more number of tillers and was taller than CO43. Also the leaves of TRY 1 were long and broad and could reach nighbouring hills or rows, offering conducive niche for web spinners to weave their webs. Ganesh Kumar and Velusamy (1996) have also reported that the activity of wolf spider *L. pseudoannulata* was greatly influenced by rice genotypes. They observed that the spider was more active in the susceptible and moderately resistant rice varieties. Hence, they recorded more mortality of rice brown plathopper (bph).

The non-webbing spider i.e. Wolf spider, *Lycosa* sp. was found to be more in CO43 (8.7) when compared to the TRY 1 (5.4), probably because of the open niche of the former which favoured the spider. Hence, it is suggested that an ecological approach involving the niche concept in conservation and utilization of natural enemies with respect to varieties are need to be considered in augmentation of spider populations in the rice ecosystem.

REFERENCES

Ganesh Kumar, M. and Velusamy, R. (1996) Integration of varietal resistance, insecticides and the predatory wolf spider, *Lycosa pseudonnulata* in the suppression of rice hoppers, *Madras Agric. J.* **83(3)**: 155–159.

Gavarra, M. R. and Raros, R. S. (1975) Studies on the biology of the predatory wolf spider, *Lycosa pseudoannulata* Boesesn and Stard (Araneae: Lycosidae)), *Philipp. Ent.* **2(6)**: 427–444.

Samal, P. and Misra, C. A. (1985) Morphology and biology of wolf spider, *Lycosa chaperi* Simon (Lycosidae): A predator of rice brown planthopper, *Oryza* **22**: 128–131.

Shivamurthappa (1993) Management of brown planthopper, *Nilaparvata lugens* (Stall) by cultural practices and insecticides, and investigation on biotypes of Karnataka. *Ph. D. Thesis*. University of Agricultural Sciences, Bangalore, 227 pp.

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Studies on Sublethal Concentration (LC_{30}) of *Annona* Seed Extract on Total Proteins of *Spodoptera litura* (Fab.)

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ABSTRACT: Influence of annona seed extract on total proteins was studied quantitatively and qualitatively in *S. litura* (Fab.). The active principles present in extract reduced the total protein content in all the stages of insect. Peak value of reduction percentage in treated was observed in II instar larvae, followed by IV instar larva. Conspicuous difference in electrophoretic pattern of proteins was observed in IV instar and adult. © 2000 Association for Advancement of Entomology

KEYWORDS: *Annona squamosa, Spodoptera litura*, proteins.

Plant derived products are known to have antifeedant, repellent and insecticidal properties and are considered to be safe due to their easy biodegradable nature. Among the 2,400 plant species other than neem, *Annona squamosa* L. leaves and seeds have insecticidal properties. The tobacco caterpillar *Spodoptera litura* (Fab.) is a major polyphagous pest of national importance and has developed resistance to some common insecticides. (Ramakrishnan *et al.*, 1984). So it is imperative to develop alternatives for control of this pest.

Annona's antifeedant activity, its influence on growth, its insecticidal properties were established. But little information is available on the impact of *Annona* on physiology and biochemical aspects of *S. litura* (Fab.). The prompted the authors to study the impact of sublethal concentration of *Annona* seed only building materials but also carries of hormones and lipids to the target which regulate the developmental stages of insects (Engelmann, 1979). Hence any effect of a xenobiotic on total proteins has physiological implication. Therefore it has been envisaged to analyze quantitatively and electrophoretically the effects of *Annona* on the total body proteins of *S. litura* (Fab.) during its development.

Petroleum either extract of *Annona* seed was prepared and its LC_{50} value was calculated for IV instar larvae of *S. litura* (Fab.) LC_{30} value (0.045%) was used in the experiment. *S. litura* culture was maintained under laboratory conditions. The larvae

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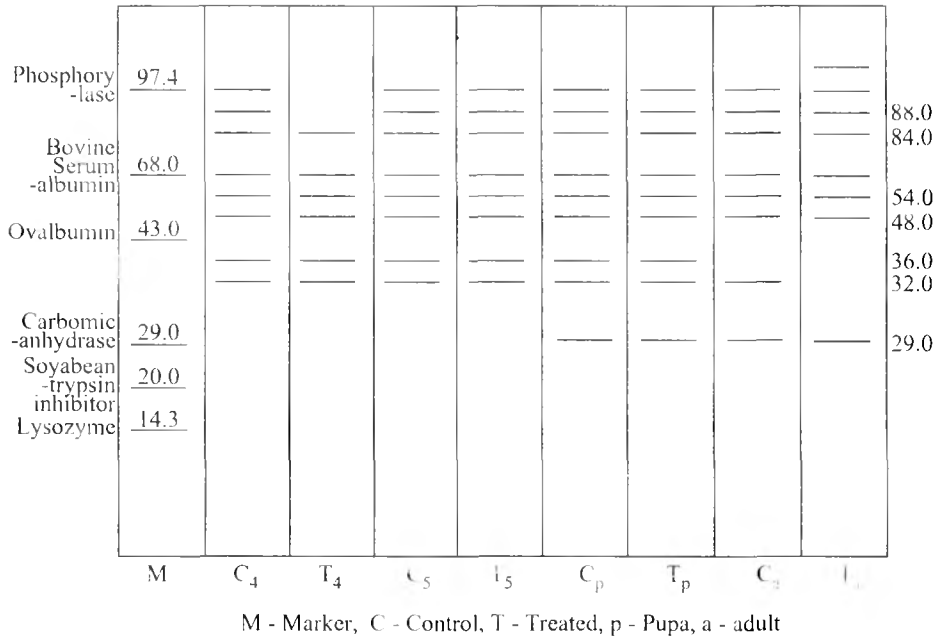

FIGURE 1. SDS-PAGE electrophoretic pattern of proteins in annona treated and untreated *S. litura* (Fab.).

fed with annona seed extract treated leaves were maintained in one lot and the other lot was supplied with leaves with distilled water. The former was considered as treated and latter as control.

The total body proteins were estimated from II instar larva to adult in both treated and untreated as described by Lowry *et al.* (1951). SDS-PAGE was used to study the protein profiles to the method of Laemmli (1970). Tissue was homogenized in phosphate buffer and centrifuged at 2000 rpm for 15 min. Supernatant was taken and 2.5 ml of 10% trichloroacetic acid was added. It was centrifuged at 2000 rpm for 15 min again and supernatant was discarded. Precipitate was dissolved in 5 ml of 0.5% Na_2CO_3. In protein estimation, this was read in colorimeter at 660 nm. and optical density (OD) values were recorded.

In SDS-PAGE, 30 μl of above solution was taken and the same volume of sample buffer was added. Samples are loaded one in each lane of stacking gel and, a current was applied at a constant voltage of 100 V. Gels were stained with 0.25% coomassie brilliant blue R. 250 in methanol : acetic acid : water (40 : 10 : 50) for 4 hours and destained using methanol : acetic acid : water (40 : 10 : 50) till the bands were visible. (Eswara Reddy and Jacobs, 2000).

Biochemical variations due to the sublethal concentration of annona seed extract in total body proteins of *S. litura* (Fab.) are presented in the Table 1 and electrophoretic pattern of proteins was shown in Fig. 1. There was high significant reduction of

TABLE 1. Effect of sublethal concentration (LC_{30}) of Annona seed extract on total protein content (mg/g) of *Spodoptera litura* (Fab.)

Treatments	Total protein content (mg/g) of *Spodoptera litura* (Fab.)					
	II instar	III instar	IV instar	V instar	Pupa	Adult
Untreated	4.06	5.25	7.08	10.09	16.75	13.74
SD	0.364	0.488	0.537	0.427	0.760	0.182
% over previous stage	–	+29.3	+34.9	+42.5	+66.0	− 18.0
With Annona seed extract	2.98	4.43	5.65	9.19	14.97	13.38
SD	0.243	0.186	0.342	0.218	0.380	0.102
% over previous stage	–	+48.6	+27.5	+62.6	+62.8	−10.6
't' value	8.190*	5.181*	7.421*	6.201*	6.924*	5.702*
% over control	−26.6	−15.64	−20.20	−8.92	−10.63	−2.62

't' table value at 0.01 – 2.319
* Significant
'+' indicates increase '−' indicates decrease
SD – Standard deviation

proteins in annona treated insects compared to untreated. Similar observations were reported by Bhagwan *et al.* (1992); Reddy *et al.* (1993); Krishnayya and Rao (1995). Reduction of total proteins due to annona seed extract might be related to reduction of protein synthesis by deranging the protein synthetic mechineray (Bharati and Govindappa, 1987a,b) or it might be due to faster rate of proteolysis (Chockalingam *et al.*, 1987).

The higher metabolic energy might be used in detoxifying the toxic principles of the extract, and lesser proportions of digested food used in synthesis of proteins might also be the reasons for reduction of total protein content in treated. Reports by Chitra and Ramakoteswara Rao (1996) are in agreement with the present results. Binding properties of proteins to foreign compounds might also be the reason for reduction of proteins, as reported by Goodman and Gilman (1965).

The per cent decrease in total protein content in the treated over control was highest in II instar larva (26.6). This could be attributed to an adaptation of insect to overcome the toxic stress at early stages and is in conformity with the findings of Anitha *et al.* (1999). The least per cent reduction in adult insect (2.62) might be due to reduction of activity of annona seed extract because of non-feeding on treated leaves by pupa and adult.

In both the treatment, total protein content was increased from II instar larva to V instar larva steadily and sudden rise in pupa followed by a fall in the adult. The fluctuation in the protein content could be correlated with the somatic growth and ovarian development. Steep raise of protein content in pupa in both treatments might be due to the synthesis of storage proteins in last larval instar and releasing of them in the pupal stage or it might be due to increase of protein concentration due to heavy loss

of body fluids in last larval instar (Tojo and Yoshiga, 1993). Reduction of total protein concentration in the adults might be due to utilization of storage proteins during pupal stage or consumption of high energy in emergence of adult from pupal case, which was supported by Gunn and Gatehourse (1987).

The results in electrophoretic pattern of proteins indicate that the total proteins were heterogeneous in size, ranging in molecular weights from 29 KD to > 98 KB. In the untreated IV instar larva, totally 8 protein bands were observed where as treated only 6 bands were observed. The missed two proteins were 98 KD and 88 KB. This could be attributed to reduction of protein synthesis in treated larva (Reddy *et al.*, 1993; Bhagwan *et al.*, 1995). This was strengthened by increase of per cent reduction of total protein content in treated over control in IV instar larva. (20.20).

Disappearance of protein bands might be due to the susceptibility of the insect for active principle of *Annona* seed extract as supported by Rajasekhara Rao and Deva Pradad (1999).

The reasons for reappearance of certain proteins in V instar larva could be essential and related to the developmental processes of the insect. No difference in proteins pattern was observed between the two treatments in V instar and pupal stages. A new protein (29 KD) appeared in the pupa of both the treatments and the bands were distinct. More number of bands and more thickness of bands in pupa might be due to sequestering of storage proteins as supported by Krishnayya and Rao (1995).

A protein with molecular weight 36 KD was not represented in both the treatments of adult insects compared to other stages and a protein of 32 KD disappeared in the treated adult. Moreover, an extra band of > 98 KD was observed in treated adult. This might be a mechanism to counteract the toxic effect of the annona active principle.

The present studies suggest that *Annona* influences the physiological processes like protein metabolism which are vital for insect life. Hence it is imperative to study on these aspects to establish it as an alternative to conventional insecticides.

REFERENCES

Anitha, B., Arivalagan, M., Nalini Sundari, M. S. and Durairaj, G. (1999) Effect of alkaloid abrine, isolated from *Abrus precatorius* (Linn.) seeds on mealy bug *Maconellicoccus hirsutus* (Green), *India J. Exp. Biology* **37**: 415–417.

Bhagwan, C. N., Pramjit Grover, Atiya Hameed and Sukumar K. (1995) Developmental defects and protein reduction caused by *Ailanthus* extract in red cotton bug, *Dysdercus koenigii*, *Indian J. Exp. Biology* **33(4)**: 287–290.

Bhagwan, C. N., Reddy, K. D. and Sukumar, K. (1992) Effect of *Annona* seed extract on protein metabolism and development in red cotton bug *Dysdercus koenigii*, *India J. Exp. Biology* **30(4)**: 908.

Bharati, D. and Govindappa, S. (1987a) Effect of carbaryl on the organic constituents in the gut region of the blister beetle *Mylabris pustulata*, *Environment and Ecology* **5(1)**: 56–59.

Bharati, D. and Govindappa, S. (1987b) Effect of carbaryl on the organic constituents of haemolymph of blister beetle *Mylabris pustulata*, *Environment and Ecology* **5(1)**: 65–70.

Chitra, K. C. and Ramakoteswara Rao, S. (1996) Effect of certain plant extracts on the consumption and utilisation of food by *Spodoptera litura (Fab.)*, *J. Insect Science* **9(1)**: 55–58.

Chockalingam, S., Nalini Sundari, M. S. and Thenmozhi, S. (1987) Proceedings of symposium, *Alternatives to Synthetic Insectivides*, CRME Madurai 99.

Engelmann, F. (1979) Insect Vitellogenin; Identification, biosynthesis and role in vitellogenesis, *Advances in Insect Physiology* **14**: 49–108.

Eswara Reddy, N. P. and Jacobs, M. (2000) Polymorphism among Kafirins and esterases in norml and Lysine-rich cultivars of sorghum, *Indian J. Genet.* **60(2)**: 159–169.

Goodman, L. S. and Gilman, A. (1965) *The Pharmacological Basis of Therapeutics*, Macmillan: New York, 21 pp.

Gunn, A. and Gatehourse, A. G. (1987) The influence of larval phase on metabolic reserves, fecundity and lifespan of the African army worm moth*Spodoptera exempta* Walker (Lepidoptera : Noctuidae), *Bull. Entol. Research* **77(4)**: 651–660.

Krishnayya, P. V. and Rao, P. J. (1995) Plumbagin effects on *Helicoverpa armigera* (Hub.) (Lepidoptera : Noctuidae). Final instar haemoloymph protein, α-amino acids and fat body protein content, *Ind. J. Exp. Biology* **33**: 217–221.

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature* **227**: 680–685.

Lowry, O. H., Rosenbrough, H. J., Farr, A. O and Randall, R. J. (1951) Protein measurement with filinphenol reagent, *J. Biol. Chemistry* **193**: 265–275.

Rajasekhara Rao, K. and Deva Prasad, V. (1999) Host-plant induced response to insecticides and haemolymph esterase pattern in American Bollworm (*Helicoverpa armigera* Hub.), *Entomon* **24(1)**: 9–13.

Ramakrishnan, N., Saxena, V. S. and Dhingra, S. (1984) Insecticide resistance in populations of *Spodoptera litura* in Andhra Pradesh, *Pesticides* **18**: 23–27.

Reddy, K. D., Bhagwan, L. N. and Sukumar, K. (1993) Depletion of aminoacids and proteins in nymphal haemolymph of the red cotton bug (*Dysdercus koenigii*) treated with *Annona squamosa* extract, *Phytophaga* **5**: 45–50.

Tojo, S. and Yoshiga, T. (1993) Purification and Characterization of three storage proteins in common cutworm (*Spodoptera litura*), *Insect Biochemistry Molecular Biology* **24**: 729–738.

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